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BLOOD PHOSPHORUS AND SERUM CALCIUM IN PARATHYROID DEFICIENCY

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In the literature on parathyroid deficiency, the phosphorus of the blood has not been determined except in the course of parathyroidectomy followed by no treatment or only in the treatment with parathormone or calcium. Even these results are few and consist very largely of determinations of the inorganic P of the blood. Under all conditions of treatment the attention in the blood chemistry has been primarily directed to its calcium content and secondarily to certain other properties.

We shall therefore devote ourselves primarily to the blood acid soluble phosphorus and reference will be made to other constituents only as they bear directly on the current problem. The reviews of Stewart and Percival (1928) on calcium metabolism, and the general reviews by Paton and Findley (1916); Boothby (1921); Biedl (1913); Simpson (1924); Carlson (1912); Jacobson (1924); MacCallum (1924); Collip (1926a); Dragstedt (1927), deal adequately with the older literature and what is at present known about the parathyroids.

Greenwald (1911) in making a series of analysis of the blood of parathyroidectomized dogs for all the important known constituents was the first to note a marked change in the P metabolism. He noted a P retention as low as 2 per cent of the normal being excreted after parathyroidectomy up to the time of tetany when the excretion was markedly increased, similar to that found in excessive exertion when an animal labors with some unaccustomed task (Engelman, 1871; Keng and Olsavsky, 1893; Paton et al., 1897). There also appeared an article by Cooke (1911) which was in complete accord with the work of Greenwald. Since then Greenwald

(1911), (1915a, b), (1916a), (1918), (1924c), and Greenwald and Gross (1925a) in a series of articles concluded that there are two and only two well authenticated findings following parathyroidectomy: a serum calcium fall and a P retention followed by a decreased excretion in Na and K. They have shown in the course of their experiments that following parathyroidectomy in dogs, (contrary to the findings of Salvesen, Hastings and McIntosh (1924)), there is no increase in Ca excretion;[1] that the phosphorus retention following a drop in the serum calcium is primary, followed by a retention in K and Na and terminating in anuria; that the phosphorus retained is largely if not entirely precipitated with Ca in the tissues; and that whatever increase in inorganic P is found in the blood is due to the breakdown of excess lipoid and protein phosphates. Total phosphorus retention and rise in blood inorganic phosphorus after parathyroidectomy have been substantiated by most investigators: Elias et al. (1922a, c); Hastings and Murray (1921); Salvesen (1923); and others (Hammett, 1924; Inouye, 1924; Frank et al., 1926).

Collip (1926b) states that the calcium falls immediately and inorganic P rises later and is found much increased in tetany. Howland (1920–21), and Freudenberg and György (1922) found a normal or increase of inorganic P in tetany. Salvesen (1924) found a slight rise in the acid soluble P of the plasma. Gross and Underhill (1922) show an increased total acid soluble P in seven dogs that developed tetany.

Hammett (1924) found an increase in the P/Ca ratio following parathyroidectomy and ascribed tetany to a disturbance in this ratio; whereas Reed, Lackey and Payte (1927) at first announced a definite mathematical relationship between the concentration of Ca and inorganic P which was progressively reduced until it was 1 at the time of tetany. Reed (1928) found after further experimentation no such definite relationship. He found that only when tetany occurs in the early post-operative period does this Ca/P ratio of 1 or less seem to hold but that at a later stage these low ratios occurred not being accompanied by tetany.

The original and subsequent work of Greenwald (1911), (1915a) on the P of the blood and excretions led him and others independently (Binger, 1917; Jeppson and Klercker, 1921; Tisdall, 1922; Elias et al., 1918, 1921, 1922b; Adlersburg and Rogers, 1923; and Collip, 1926) to investigate the action of phosphates by the injection of various phosphate salts into the blood. The fact that various cations were used with the PO_4''', HPO_4'', H_2PO_4' anions brought various other cation and anion relationships as well as the acid base balance into question, not only in parathyroid tetany but in all other forms of tetany. This question with all the literature has been most excellently reviewed by MacCallum (1924). To this may be

[1] Some of the authors' unpublished work is in accord with Greenwald's findings.

added the later reviews of Stewart and Percival (1928) and the earlier papers of Greenwald (1918) (1922). Full consideration of the subject is out of the scope of this paper.

On the other hand, parathormone overdosage both in normal and parathyroidectomized animals bring about a marked rise in inorganic P which is secondary to a marked calcium rise to above the normal value (Fisher and Larson, 1925; Collip, 1926a, c).

Salvesen (1925) observed in parathyroidectomized dogs and cats a decrease in inorganic P (6 or 7 to 4 mgm. per 100 cc.) with a terminal rise as in the normals. Collip (1926) found in parathyroidectomized rabbits that with the Ca almost normal, the injection of parathormone may bring about a rapid high inorganic P accompanying fatal tetany. They found increased excretion of Ca and P in the urine. These results on parathormone were affirmed by Greenwald and Gross (1925c), (1926); and Grollman (1927). It can thus be concluded that given a low Ca level, the effect of parathormone on the blood inorganic P is opposite to that caused by parathyroidectomy.

With the view of possibly obtaining more insight into the relationship between blood P and parathyroid function the present work was undertaken. The points investigated were the inorganic phosphorus and the total acid soluble phosphorus of the blood in normal and thyroparathyroidectomized dogs, and these same animals treated variously to prevent or abolish tetany. The serum calcium was determined in every case for comparison to the blood phosphates, and in particular to serve as an index to the amount of parathyroid deficiency produced.

METHODS OF EXPERIMENTATION. After an adequate control period, dogs were completely thyroparathyroidectomized. We found that if the thyroids were carefully removed including all the immediately surrounding fatty tissue and ligating and cutting the thyroid vessels as close to the carotid as possible, 100 per cent of the animals developed symptoms of parathyroid deficiency from which they died. In our series the last 50 dogs were thus operated and out of these 50 we were unable to keep a single dog in a healthy condition or from death with continuous treatment even with calcium lactate (153 days) or after prolonged treatment with parathormone.

For the prevention of fatal tetany the dogs were then treated by well established methods: *a*, calcium lactate (9 dogs); *b*, strontium lactate (2 dogs); *c*, magnesium lactate (2 dogs); *d*, morphine sulphate (2 dogs); *e*, ammonium chloride (4 dogs); *f*, uranium nitrate (9 dogs); *g*, parathormone (11 dogs). In addition 23 other dogs were treated with (heparmone) liver extracts.[2] Twelve dogs were allowed to run their course without

[2] The results on liver extract and parathormone treatment are published in separate papers.

treatment. Of these 76 dogs, 42 (including the 12 that never received treatment) received no treatment until symptoms of parathyroid deficiency appeared, whereas 34 received treatment from the day following that of the operation.

The calcium, strontium, magnesium and ammonium were given by stomach tube, usually in 5 per cent solution, 4 to 12 grams, twice daily, 3 to 5 hours apart, followed by a meal 2 to 3 hours later or as described by Wenner (1926 and 1927), and Dragstedt and Sudan, (1926a, b) 2 to 3 hours before and 4 to 5 hours after a meal. Morphine was given intravenously or subcutaneously in 0.5 to 3 grain doses never exceeding 5 grains a day according to the method of Sloan (1926). The uranium nitrate was given subcutaneously in single 0.3 to 5 mgm. doses per K. body weight according to the apparent age of the animal at the time of tetany as described by Wenner and Swingle (1926).

The blood analysis throughout the control and experimental period was made from 1 to 4 and even 7 times a day, daily, 2 to 5 times a week, weekly, biweekly, etc., according to the nature of the results and the method of treatment employed. The blood analysis extends over the experimental period of each dog lasting from several days to weeks, months and even a year.

The blood was obtained directly from the heart. The serum calcium was determined by the method of Kramer and Tisdall (1921) as modified by Clark and Collip (1925). The inorganic and total acid soluble phosphorus (T.A.S.P.) of the whole blood was determined by the method of Subbarow and Fiske (1925).

In all the tables, the calcium is reported in milligrams per 100 cc. of serum (mgm. per cent) and the inorganic P. and T.A.S.P. in milligrams per 100 cc. of whole blood (mgm. per cent).

Normal P and Ca concentrations. The data include 261 determinations made on 71 dogs under normal conditions. The average of all the normal determinations was 10.84 mgm. per cent for the serum calcium, 4.44 mgm. per cent for inorganic P, 26.65 mgm. per cent for T.A.S.P; 0.41 for the inorganic P/Ca ratio. The serum calcium is quite constant from time to time within the same animal and as a rule from animal to animal—however, 3 dogs had a serum calcium level that was constantly in the neighborhood of 8.75 mgm. per cent and another dog had a constant high serum calcium of 12.55 mgm. per cent. The inorganic P varies markedly from time to time (up to 2 mgm. per cent) and from animal to animal (up to 2.5 mgm. per cent). The T.A.S.P. also varies from time to time (usually 1 to 2 but maybe up to 6 mgm. per cent) and from animal to animal (up to 14 mgm. per cent). Due to the above both the inorganic P/Ca (0.29 to 0.57) and the T.A.S.P./Ca (1.83 to 3.44) ratios may vary markedly from animal to animal but less so from time to time within the same animal.

However, taking the average of the determinations made on each dog and comparing this to the average values found for the 71 dogs, the variation is much less and hardly ever exceeds ±0.3 mgm. per cent for the serum calcium, ±0.5 mgm. per cent for the inorganic P, ±0.05 for the inorganic P/Ca ratio, and ±0.3 to 0.35 for the T.A.S. P/Ca ratio.

TABLE 1

The whole blood inorganic P, and total acid soluble P, serum calcium in milligrams per cent, and their respective ratios in normal and thyroparathyroidectomized dogs when symptoms of parathyroid deficiency appeared (see immediate text for explanation of parts)

NUMBER OF DOGS	DAY OF SYMPTOMS	SERUM Ca (MGM.)		INORG. P (MGM.)		T.A.S.P (MGM.)		INORG. P/Ca		T.A.S.P./Ca	
		Normal	Symptoms	Normal	Symptoms	Normal	Symptoms	Normal	Symptoms	Normal	Symptoms
					I. Dogs showing sudden and severe tetany						
8	2	11.20	5.99	4.60	5.56	27.76	29.95	0.41	0.99	2.45	5.00
1	3	10.53	4.81	3.18	3.92	23.40	23.67	0.30	0.81	2.22	5.12
1	4	10.52	6.62	3.20	4.73	25.13	33.18	0.30	0.71	2.38	5.01
1	5	10.12	5.13	3.54	7.27	22.84	27.40	0.35	0.15	2.25	5.34
11	Average	10.59	5.66	3.84	5.34	24.78	28.55	0.24	0.92	2.33	5.12
					II. Dogs showing mild tetany						
2	1	10.56	7.68	3.48	5.51	27.54	28.59	0.33	0.72	2.61	3.72
15	2	11.01	6.43	4.64	6.67	26.92	27.52	0.42	0.88	2.45	4.28
4	3	10.67	6.98	4.41	5.41	26.46	25.23	0.41	0.90	2.48	4.22
2	4, 5	10.39	5.64	4.13	4.81	25.76	24.85	0.40	0.85	2.48	4.41
23	Average	10.90	6.35	4.48	5.60	26.67	26.55	0.39	0.86	2.45	4.25
					III. Dogs showing depression						
1	1	10.43	7.84	3.62	4.14	19.12	24.37	0.35	0.53	1.83	3.10
5	4, 5	10.31	6.02	4.17	5.53	27.09	26.55	0.40	0.92	2.63	4.41
2	6	11.73	6.95	4.58	5.54	25.05	22.21	0.39	0.80	2.14	3.20
8	Average	10.68	6.48	4.20	5.35	25.58	26.42	0.39	0.83	2.40	4.08

P and Ca concentration when symptoms of parathyroid deficiency appeared. In studying the data of the 42 dogs that were not treated until symptoms of parathyroid deficiency appeared, we were impressed with the changes in blood P and serum Ca content that characterized dogs showing sudden and severe tetany, mild tetany and only a prolonged stage of depression. We noticed also that these changes in blood phosphorus and serum calcium content were affected by the number of days

that elapsed between the operation (thyroparathyroidectomy) and the onset of symptoms of parathyroid deficiency.

Accordingly, the data on these 42 dogs have been arranged in the form of a table (table 1). It shows the total acid soluble P and inorganic P of the whole blood, the serum Ca in milligrams per cent and their respective P/Ca ratios according to the post-operative day when symptoms of parathyroid deficiency first appeared and the number of dogs showing such symptoms on the given symptomatic day. It is again divided into 3 parts representing 3 groups of dogs: I, showing 11 dogs that, so to speak, without any very apparent warning suddenly fell into a severe attack of tetany; II, showing 23 dogs developing a mild state of tetany slowly; and III, showing 8 dogs developing a progressive increase in depression with no apparent tetany. The last line of each part shows the average for its respective part of the table and may serve as a comparison between the 3 groups of dogs.

It will be noted that the table presents only the calcium, phosphorus and the P/Ca ratios at the time of operation (average of all normal determinations) and at the time when symptoms appeared. It would unnecessarily complicate the results to include the data of determinations made from the time of operation to the time when symptoms of parathyroid deficiency appeared.

Whereas the calcium fell progressively from the time of the operation until symptoms appeared, there were no such constant changes for the phosphorus compounds of the blood. The P/Ca ratios always tended upwards from the time of the operation. Both inorganic and total acid soluble phosphorus varied widely during the presymptomatic period. The inorganic and total acid soluble phosphorus in the whole blood may be high within 24 hours after the operation (due to the anesthetic) and then fall progressively until symptoms appear, or just the reverse takes place. All gradations may be found between these extremes.

The inorganic and total acid soluble phosphorus did by no means always vary in the same direction. Again, though there be a rapid rise in the P/Ca ratios post-operatively, symptoms of parathyroid deficiency do not always occur when the disturbance in either or both of the P/Ca ratios is at a maximum. This is more often true when symptoms do not develop until several days after the operation. As a rule, however, these ratios are in the neighborhood of the maximum disturbance when symptoms first appear. But dogs surviving a few attacks of tetany show no direct constant relationship to these ratios in their symptomatology as we shall see in table 2, dog 69.

Total acid soluble phosphorus when symptoms appeared. The total acid soluble phosphorus finds its widest variation and greatest rise in the severe tetany dogs (table 1, part I). With one exception every dog in this

group showed a higher than normal total acid soluble phosphorus when symptoms appeared, no matter whether tetany occurred on the second or fifth post-operative day. The rise was from 1 to 8 mgm. per cent in individual dogs. The severity of tetany was not identical and proportional to the change of phosphorus content. The change in phosphorus content precedes, accompanies or follows the appearance of symptoms.

The dogs in the second group differed from those in the first group in that the phosphorus variations were not so extensive in the former and in that the phosphorus values were below the normal when the onset of mild tetany was delayed for several days after the operation. Only in one dog was there a rise of 6 mgm. per cent of total acid soluble phosphorus. The remaining mild tetany dogs showed only a slight rise (1 to 2 mgm. per cent) or an actual decrease in blood total acid soluble phosphorus (2 to 6 mgm. per cent). In this group the phosphorus is actually the same or below the normal when symptoms appear on the third, fourth and fifth days and even in some on the second day. The depression dogs show about the same phosphorus variations as the dogs in part II.

Comparing these three groups of dogs as a whole it is significant that only the severe tetany dogs show a marked average increase of total acid soluble phosphorus above the normal at the time that symptoms appeared whereas the mild tetany and depression dogs showed very little change.

These considerations would lead us to conclude that in a large series of dogs the total acid soluble phosphorus tends to increase as the result of the increased activity found in parathyroid tetany were it not for the fact that dogs developing mild tetany or severe depression on the first or second day following the operation show the same rise in the total acid soluble P. Moreover, dogs not developing mild tetany or depression until several days after the operation do not show this increase of phosphorus. The latter to the contrary usually show a few milligrams fall in the T.A.S.P. below the normal. Apparently, there is no definite level for the total acid soluble P at which symptoms of parathyroid deficiency appear—symptoms appearing at levels as low as 19.5 mgm. per cent and as high as 36.6 mgm. per cent.

Inorganic phosphorus when symptoms appeared. The inorganic phosphorus of the whole blood for these 3 groups of dogs are represented in the same respective portion of the table that has been discussed for the T.A.S.P. About all that can be said of the inorganic phosphorus is that without exception the concentration is always higher than normal when symptoms appear. It does not tend to exhibit the relationship to the severity of the symptoms and the time of their onset that the total acid soluble phosphorus does. The symptomatic values of inorganic P are from 0.25 to 2.5 mgm. per cent above the normal concentration.

Serum calcium when symptoms appeared. With few exceptions the

calcium is constantly in the neighborhood of 5 to 7 mgm. per cent when symptoms of parathyroid deficiency appeared in untreated dogs. Our data are of interest in that the average values for the 3 groups at the time that symptoms appeared are within 0.85 mgm. per cent of each other:

Group I, 4.23 to 7.44; average 5.66
Group II, 5.06 to 7.93; average 6.35
Group III, 4.55 to 7.84; average 6.48

However, the calcium range at which symptoms (no matter how mild or severe) may appear is just as great for one group of dogs as it is for another, nor does it depend on the post-operative time when symptoms appear. It seems that the calcium tends to fall progressively from the time of operation and that it is the rate of fall that determines the onset of symptoms. That is to say, a dog showing a slow decrease in serum calcium in the course of several days, as in the case of dog 69 (table 2), can withstand a much lower calcium than a dog that shows the same decrease in the first day or two. However the rate of calcium change does not determine the type or severity of symptoms that may appear. Thus one dog showed the same severe tetany on the second post-operative day at 7.44 mgm. per cent Ca or a second dog at 4.23 mgm. per cent Ca as did another dog on the fifth day at 5.13 mgm. per cent Ca. Similar comparisons may be made for the mild tetany dogs and the depression dogs.

Although this progressive lowering of serum calcium is the rule, the mild tetany and depression dogs showing symptoms beginning with the fourth to the sixth post-operative day have a slightly higher calcium than a day or two previous to the time that symptoms appear.

Total acid soluble phosphorus Ca ratio when symptoms appeared. This ratio is highest for group I (4.28 to 6.50; average 5.12); next comes group II (3.25 to 5.46; average 4.25), followed closely by group III (2.46 to 6.04; average 4.08). This is just what one would expect since the total acid soluble phosphorus tends to increase directly with the severity of the symptoms manifested and decreases with the increase in time of their post-operative onset.

Hence, within any of these respective groups the high total acid soluble phosphorus in early and severe symptoms when the serum Ca is still relatively high will tend to equalize the ratio to that found in dogs showing symptoms several days later when the total acid soluble phosphorus has already fallen below the normal, but the calcium is relatively lower because of its continued progressive fall from day to day. But since the progressive fall of calcium is relatively greater than the later decrease of total acid soluble phosphorus in all three groups of dogs, the total acid soluble phosphorus/Ca ratio remains the same or shows a slightly higher value in dogs showing symptoms in later days within the same group (table

1). This table shows that the ratio is highest for the severest tetany dogs and lower for the mild tetany and depression dogs (last line of each part in table 1). I, II and III show that as one goes from day to day when symptoms were manifested the value of the ratio remains either the same or is slightly increased.

Inorganic P/Ca ratio when symptoms appeared. From the table (3) it is obvious that the inorganic P/Ca ratio tends to parallel the total acid soluble phosphorus/Ca ratio; however the extent of change for the inorganic P/Ca ratio is not as constant as that of the total acid soluble P/Ca ratio.

Conclusions. We may thus conclude that the total acid soluble phosphorus (whole blood content relative to its normal concentration) of untreated parathyroidectomized dogs when symptoms of parathyroid deficiency appear tends to increase directly with the severity of the symptoms manifested; however, if the symptoms are mild and delayed in onset for 3 or more days there is usually a fall to below the normal; that under the same conditions the inorganic phosphorus is always above normal when symptoms appear but frequently it does not parallel the T.A.S.P. changes; that the rate of serum calcium fall and not so much the absolute content determines the onset of parathyroid deficient symptoms but bears no relationship to the type and severity of symptoms manifested: that the total acid soluble P/Ca ratio tends to vary directly with the severity of the symptoms manifested and the time of their post-operative onset: and that the inorganic P/Ca ratio tends to parallel the T.A.S.P./Ca ratio exhibiting less constancy in its extent of change.

P and Ca concentrations of untreated parathyroidectomized dogs at the time of (1) death or (2) recovery. Of the 12 untreated parathyroidectomized dogs that showed symptoms, there were ten. Eight of these died: 2 on the second, 3 on the third, 2 on the seventh and 1 on the eighth post-operative day. All of these dogs showed more or less severe symptoms from the second post-operative day. The other 2 dogs showed mild tetany and severe depression symptoms but not until the seventh day. Recovery occurred on the 16th (table 3) and 34th days, respectively. One of these dogs, a female, was later injected with parathormone, developing parathyroid deficiency symptoms subsequent to discontinued treatment, from which she died; the other, a male is still in excellent condition now, 16 months after operation and more than 15 months after complete recovery.

1. Ca and P values at time of death. The results from these dogs are shown in table 2. It is evident from these results that the conclusions drawn above for the changes taking place until symptoms of parathyroid deficiency appear apply equally well to the changes taking place in the blood from the time that the symptoms appear until death.

Thus the total acid soluble phosphorus of the dogs that died about 2

hours after sudden development of severe tetany on the second post-operative day indicate a rise of 2 mgm. per cent to the time that symptoms appear and an additional 5 mgm. per cent just preceding death; those dying on the third day, a fall of 1.5 mgm. per cent at the time of symptoms and then a rise of 6 mgm. per cent just before death, those dying on the seventh day, a 2 mgm. per cent rise at the time of symptoms and a fall of 6 mgm. per cent at the time of death; whereas the dog on the eighth day showed a 2 mgm. per cent fall to the time that symptoms appeared and an additional fall of 1.7 mgm. per cent at the time of death.

The inorganic P shows a similar change to that of the total acid soluble phosphorus but is never below its normal concentration at the time of

TABLE 2

Showing eight dogs that died of parathyroid deficiency without treatment

All dogs showed symptoms on second post-operative day. Table is arranged according to day of post-operative death—showing concentration of serum Ca, whole blood inorganic and total acid soluble phosphorus and the P/Ca ratios when normal, when symptoms appeared and at time of death.

NUMBER OF DAYS	DAY OF DEATH	SERUM Ca (MGM.)			INORG. P (MGM.)			T.A.S.P. (MGM.)			INORG. P/Ca			T.A.S.P./Ca		
		Normal	Symptoms	Death	Normal	Symptoms	Death	Normal	Symptoms	Death	Normal	Symptoms	Death	Normal	Symptoms	Death
2	2	11.50	5.84	6.52	4.18	4.92	7.00	27.81	29.68	34.94	0.37	0.92	1.13	2.45	5.37	5.68
3	3	11.51	6.23	5.46	5.81	6.02	7.58	29.40	28.03	33.16	0.50	0.94	1.41	2.57	4.38	6.09
2	7	10.50	5.20	4.77	5.62	5.75	4.00	27.79	29.91	23.50	0.35	1.12	0.84	2.38	5.72	4.95
1	8	11.12	7.23	4.42	4.40	5.16	5.20	25.10	23.20	21.55	0.39	0.71	1.17	2.25	3.20	4.87
8	Ave.	11.15	6.07	5.46	4.50	5.58	6.26	27.53	28.60	29.74	0.40	0.92	1.15	2.41	4.67	5.51

death, although in one instance it is below the concentration of the first attack of tetany at the time of death.

The serum calcium falls more or less progressively from day to day until death, although it may sometimes be lower between convulsions than at the time of severe tetany. The dogs that died with the first sudden and acute attack and one dying on the seventh day showed a rise in serum calcium just preceding death, amounting to 1 mgm. per cent.

Both total acid soluble and inorganic P/Ca ratios were higher at the time just preceding death than at the time when symptoms appeared except in one dog dying on the seventh day. His ratio fell so markedly on the day of death due to a calcium rise (4.05 to 5.17) that in table 2 it would seem that both dogs dying on the seventh day had lower ratios at the time of death than at the time when symptoms appeared. Obviously, however, the relationship between the time of post-operative death and

TABLE 3

Showing dog 69 who recovered without treatment and is now in excellent condition, 16 months after operation

Dog 69, male. Ca in milligrams per 100 cc. of serum. Inorganic and total acid soluble P in milligrams per 100 cc. whole blood.

DATE	TIME	REMARKS	SERUM Ca	INORG. P	T.A.S.P.	INORG./Ca	T.A.S.P./Ca
1928							
February 27	3:00 p.m.		10.59	4.39	24.54	0.44	2.32
February 28	4:00 p.m.		11.07	4.52	24.85	0.41	2.24
March 1	6.00 p.m.		11.42	4.54	24.47	0.40	2.14
March 2	5:20 p.m.		11.52	4.61	24.18	0.40	2.10
March 6	5:00 p.m.		11.71	4.69	25.30	0.40	2.11
March 7	4:40 p.m.	Thyroparathyroidectomy					
March 8	3:00 p.m.		9.81	5.35	26.44	0.54	2.69
March 9	2:00 p.m.		8.03	4.97	24.97	0.62	3.11
March 10	10:00 a.m.	No symptoms	7.08	4.88	24.28	0.69	3.43
March 11		Not fed					
March 12	2:00 p.m.	No symptoms of tetany	7.06	5.87	25.66	0.83	3.63
March 13		Did not eat					
March 14	3:00 p.m.	Twitches. Meal by stomach tube. Vomits $\frac{1}{3}$	6.03	5.47	24.78	0.91	4.11
March 15	9:00 a.m.	Twitches. Meal by stomach tube. Vomits $\frac{1}{2}$	5.52	5.13	26.14	0.93	4.37
March 16	1:40 p.m.	No twitches. Meal by stomach tube. Depressed	5.31	5.34	26.14	1.01	4.92
March 17	11:50 a.m.	No twitches. Depressed. Meal by stomach tube. Vomits $\frac{1}{2}$	5.11	5.33	27.95	1.02	5.47
March 18	1:00 p.m.	No twitches. Depressed. Meal by stomach tube.	5.16	6.00	30.30	1.16	5.87
March 19	3:30 p.m.	No twitches. Depressed. Meal by stomach tube	4.56	5.72	31.97	1.26	7.01
March 20	3:45 p.m.		5.32	5.52	30.80	1.04	5.79
March 21	3:30 p.m.		5.29	5.79	31.37	1.09	5.93
March 22	3:35 p.m.		5.00	5.63	30.12	1.13	6.02
March 26	4:00 p.m.	Eats own meal	5.16	5.26	25.67	1.02	4.97
June 9		Growing fatter	10.44	5.34	33.25	0.51	3.19
June 10		Weight 13.4 kgm.	10.62	4.94	31.67	0.47	2.98
June 22			10.72	4.52	28.68	0.42	2.68
July 3	3:15 p.m.	Excellent condition	7.85	4.57	30.44	0.58	3.88
July 4	10:15 a.m.	Quite well	8.15	4.98	29.73	0.61	3.65
July 19	10:00 a.m.	Excellent condition	7.67	5.10	31.90	0.66	4.16
October 9	10:30 a.m.	Dog in excellent shape. Wt. 13.4 kgm.	7.62	5.19	29.00	0.68	3.81
October 16	11:00 a.m.	Dog in excellent shape	7.22	4.91	28.55	0.68	3.95
October 25	11:00 a.m.	Dog in excellent shape	7.13	5.03	30.06	0.71	4.22

TABLE 3—*Concluded*

DATE	TIME	REMARKS	SERUM Ca	INORG. P	T.A.S.P.	INORG./Ca	T.A.S.P./Ca
1928							
November 15	12:00 a.m.	Dog in excellent shape	7.43	5.05	28.92	0.68	3.89
November 23	2:00 p.m.	Dog in excellent shape		5.44	29.65		
December 7	10:30 a.m.	Excellent shape		4.44	28.35		
1929							
January 13	2:00 a.m.	Excellent shape	7.62	4.30	29.20	0.56	3.83
January 22	2:30 p.m.	Excellent shape	7.93	4.56	27.87	0.58	3.51
February 9	3:00 p.m.	Excellent shape	8.45	4.37	27.40	0.52	3.24
February 23	1:00 p.m.	Excellent shape	8.92	4.08	25.30	0.46	2.84

the maximum disturbance of these ratios is not so striking. For instance in one dog these ratios varied between 5.62 and 6.50 (T.A.S.P./Ca) and 0.75 and 1.20 (inorganic P/Ca) from the time of tetany on the second day until death on the seventh day, with death finally occurring at the respective ratios of 4.73 and 0.82. On the other hand dog 69 (table 3) did not die and his respective ratios rose as high as 7.01 and 1.26. Apparently the high P/Ca ratios are not the cause of death.

2. Ca and P values during and after recovery. Dog 69 (table 3) now living 16 months after operation and in good condition showed severe depression and mild tetany and was fed daily during this period (by stomach tube) the regular maintenance diet, consisting of powdered dried beef heart, ground cracker meal, kaolin and corn oil and a definite amount of water. Thus this dog even with a balanced protein diet without milk and no treatment was able to withstand a calcium as low as 4.56 and an inorganic P and T.A.S.P., respectively, of 6.00 and 31.97 with ratios of 1.26 (inorganic P/Ca) and 7.01 (T.A.S.P./Ca). Even after 10 such days when convalescence began the respective values in the order named above were 5.16; 5.26; 25.67; 1.02; and 4.97.

A month later the animal was returned to the regular stock diet of bread, hamburger and some milk and has never since shown marked symptoms of parathyroid deficiency. In the period from January 9, 1928 to February 23, 1929, his calcium varied from 7.13 to 10.72 mgm. per cent; his inorganic P from 4.08 to 5.44 mgm. per cent; his T.A.S.P. from 33.25 to 25.30 mgm. per cent; his inorganic P/Ca ratio from 0.42 to 0.71; and his T.A.S.P./Ca ratio from 4.22 to 2.84. There is a marked tendency to reëstablish the normal ratios, either by lowering the P content or raising the calcium or both. This latter fact was also seen in another dog where on recovery the Ca rose from 5.33 to 8.20 mgm. per cent; the inorganic P and T.A.S.P. fell, respectively, from 7.02 to 5.16 and 27.20 and 19.80 mgm. per cent

while the ratios were reduced from 1.32 to 0.62 (inorganic P/Ca) and 5.14 to 2.41 (T.A.S.P./Ca).

The above results indicate strongly that the low serum calcium level or high phosphorus level are not the only factors involved in producing tetany and death in parathyroid deficiency.

Here were 2 dogs that received absolutely no treatment at all, were both fed a balanced protein diet, had a low serum calcium, high acid soluble phosphorus compounds accompanied by depression, mild tetany and laryngismus stridulus—in fact they had all the indications of a parathyroid deficiency that ordinarily leads to fatality—and still the animals recovered.

It is thus difficult to conclude just what rôle any of the factors discussed may play in the symptomatology and events leading to death in a parathyroid deficient animal. It seems that in the events leading to early death the rate of calcium and phosphorus change may play a part, whereas in animals dying or recovering after several days of symptoms it becomes a question of maintenance of life at a progressively lower absolute calcium content until the body is able to compensate more fully for the parathyroid deficiency. A low blood inorganic and particularly total acid soluble phosphorus content is highly favorable in bringing about this compensation or readjustment.

Our findings on the blood inorganic phosphorus in dogs suffering from parathyroid deficiency agree with those reported by other investigators, namely, that it increases, however, inconstantly and varies considerably in amount (Greenwald, 1913, 1924a to c; Salvesen, 1923, 1924; Gross and Underhill, 1922). It is possible in some cases that the changes observed in both total acid soluble and inorganic P may have been due to kidney injury or even to ether anesthesia (especially in those where a marked increase in both was observed only the first post-operative day) since this has been found to increase the inorganic phosphate of the blood in normal rabbits (Martland and Robison, 1924) and also in dogs (Stehle and Bourne, 1924).

Dogs treated with calcium lactate. The immediate relief from parathyroid tetany on injection of solutions of calcium salts was demonstrated by Parhon and Ureche (1907) and later by MacCallum and Voegtlin (1909). This has been substantiated by all later investigators.

Luckhardt and Goldberg (1923) by oral administration of Ca lactate and subsequently Compere and Luckhardt (1924) by oral administration of $CaCO_3$, $Ca(NO_3)_2$ and $Ca(C_2H_3O_2)$ kept dogs alive and in good condition following a complete thyroparathyroidectomy whereas monobasic calcium phosphate seemed more likely to induce tetany rather than to prevent it. Dragstedt and Sudan (1926) confirmed these results and pointed out the increased dosage necessary to prevent tetany in young dogs and pregnant animals.

We found that dogs may recover from the symptoms of parathyroid

deficiency after at least 60 days of treatment by calcium lactate, orally. The treatment must be started before the animal exhibits severe tetany. Two dogs receiving treatment from the time that symptoms appeared died on the 3rd and 6th day, respectively, while a third dog that was pregnant at the time of operation received 103 days of treatment (46 days before and 57 days after parturition) and was killed 2 weeks later because of a severe skin rash. Three dogs receiving treatment from the day following the operation died on the 18th, 112th, and 153rd days, respectively, and received treatment 18, 104 and 60 days, respectively.

Under calcium therapy of parathyroid deficient dogs the behavior of the serum calcium, whole blood inorganic and total acid soluble phosphorus and the P/Ca ratios whenever parathyroid deficient symptoms appeared and at the time of death was identical to that shown for dogs without any treatment.

Under calcium therapy of parathyroid deficient dogs, the inorganic P and T.A.S.P. have a tendency to be below the normal so long as the animal is benefitted and so long as the calcium concentration is not markedly above the normal. When the animal is not benefitted by the calcium administration or when the calcium is raised markedly above the normal, both the inorganic P and the T.A.S.P. increase. In the former condition (tetany) the rise in the latter is more constant and in the latter (above normal) the rise in the former is more constant.

The P/Ca ratios are more or less a function of the serum calcium concentration—that is, the changes in serum Ca are so marked by calcium administration that they hide the lesser change in the numerator and therefore vary inversely with the change of the denominator.

Dogs treated with strontium lactate. In 1909 Berkley and Beebe first reported the beneficial effect of strontium salts in the relief of parathyroid tetany but stated that animals thus benefitted died in a few days of nutritional disturbances. A year later Voegtlin and MacCallum (1910) also identified strontium with calcium in its relief of parathyroid tetany. This work has been repeated and extended by Dragstedt and Sudan (1926b) and Swingle and Wenner (1926). The latter two claim from their experiments on 27 dogs that strontium given orally is effective in bringing about recovery from parathyroid deficiency in the course of 3 weeks.

In our own hands strontium lactate treatment was not nearly so effective as calcium lactate in bringing about recovery from tetany without depression or in saving the life of the animal. The dogs died on the 10th and 13th post-operative days but not in tetany.

As in the case of calcium lactate treatment so it is with strontium; so long as the animal was benefitted by the treatment, the inorganic P and T.A.S.P. remained the same or decreased. However, as the symptoms that led to death increased, the T.A.S.P. rose by 6 to 7 mgm. per cent while the inorganic P varied.

It is felt that since the method used for Ca determination includes also that for strontium (Fay, 1926), this is the cause for the high calcium values found at death. The high calcium values would thus cause low or very nearly normal P/Ca ratios even if there is a marked rise in the T.A.S.P.

It is suggested that the high calcium value, though it abolished tetany, cannot replace Ca in its biological relationship since death followed just the same.

Dogs treated with magnesium lactate. Berkley and Beebe (1909) and Voegtlin and MacCallum (1910) demonstrated the rapid disappearance of the neuromuscular disturbances in parathyroid tetany by the use of magnesium salts. This work was affirmed and extended by Luckhardt, Waud and Brannon (1926) who kept parathyroidectomized dogs free from tetany for a period of 6 days when the serum calcium was as low as 4 mg. per cent. Wenner (1927a) found that after 27 to 73 days of magnesium treatment, dogs recover from the symptoms of parathyroid deficiency if parathyroidectomized dogs are never allowed to go into tetany. He suggests that magnesium when in excess takes care of the increased phosphates or in some way brings the calcium back into solution.

In our own hands the lives of 2 dogs could not be maintained beyond 11 and 23 days, respectively, by magnesium lactate treatment.

The results indicate that in parathyroidectomized dogs magnesium treatment neither prevents nor promotes a decrease in the serum calcium. The results also indicate (contrary to the supposition of Wenner, 1926, 1927) that the magnesium does not exert its beneficial influence to a great extent, by making calcium more available as a result of replacing the latter from its phosphate combination, since the serum calcium falls progressively (no faster than in the normal) and the inorganic P and T.A.S.P. are both markedly increased after the second day even in dogs completely narcotized by magnesium. But since the phosphorus compounds do not rise until the second or third day after magnesium treatment is begun it appears that the phosphorus rise may be due to the lower calcium or the effects of high magnesium content, and that therefore initially the magnesium may act by replacing the Ca from its phosphate combination, but certainly not later.

Thus, whatever other relationship there may be between magnesium and other ions in the body, its beneficial effects in parathyroid deficiency are largely, if not entirely, due to its generalized nervous and metabolic depressant effect.

Dogs treated with morphine sulphate. The experiments of Sloan (1926) were repeated and his results affirmed. He observed the beneficial effects of morphine in the treatment of parathyroid tetany and observed that it had no effect on the serum calcium.

We found it impossible to keep dogs on morphine treatment more than 5 successive days because of its systemic effects.

The effect of prolonged morphine sulphate treatment on the blood picture is very nearly identical to that produced by magnesium except that the inorganic phosphorus rises to a greater degree, particularly terminally, and the serum calcium varies within 1 mgm. per cent of the level found when the treatment is begun. It seems as if the calcium is kept from decreasing farther by morphine treatment whereas magnesium did not have this property. Also the total acid soluble phosphorus rises earlier in morphine treatment than it does in magnesium. Whenever calcium lactate was given, orally, the phosphorus compounds decreased and reached as far as 3 to 8 mgm. per cent below the normal in T.A.S.P. content whereas the inorganic P was less affected and never even decreased to the normal concentration.

Since the calcium remains so very nearly the same throughout the period of morphine treatment it is evident that the direction and extent of change of both P/Ca ratios will be dependent on and nearly directly proportional to the change in concentration of the respective P compounds. Thus the ratios increase progressively until death.

The beneficial effects of morphine have been attributed to its depressant action on the central nervous system and its effect on respiration by, primarily, stimulation when it has become paralyzed and, secondarily, depressing thus preventing the alkemia of hyperpnea as suggested by Sloan (1926).

Dogs treated with ammonium chloride. It seems that dogs may be freed from tetany by the oral administration of calcium lactate followed by ammonium chloride and still not survive. Such dogs die in a curled up position as if suffering from cold. Previous to death they become weak and emaciated, their vomit contains chocolate colored blood and they have a watery diarrhea. In such dogs the tendency toward hemolysis is strong, the serum calcium may be normal or below the tetany level with an inorganic P that is 2 mgm. per cent below to 3.5 mgm. per cent above the normal and a T.A.S.P. that has been reduced to 12 mgm. per cent or raised by 9 mgm. per cent at the time of death.

Only one dog was brought to complete recovery by ammonium chloride treatment (33 days) and died in tetany 6 days later. Two other dogs were benefited for 3 and 7 days, respectively. Another dog died the first day without tetany.

All four dogs treated with ammonium chloride show that the ammonium chloride has a tendency to keep or raise the serum calcium above the tetany level. In the 3 dogs benefitted by the treatment the inorganic P (1 to 2.5 mgm. per cent) and T.A.S.P. (6 to 14 mgm. per cent) are markedly reduced. Both phosphorus compounds increase correspondingly upon de-

crease or withdrawal of ammonium chloride treatment regardless of the serum calcium content. The behavior of the phosphorus compounds is therefore due to the NH_4' itself and not through the agency of affecting the serum calcium.

Both the T.A.S.P./Ca and inorganic P/Ca ratios decrease markedly, even to below the normal, when the animals are benefitted by ammonium chloride treatment and vice versa when the treatment is withdrawn.

Our results confirm those of Wenner (1927b) that the ammonium chloride probably exerts its action by rendering the blood more acid, thereby producing a rise in serum calcium. But since the behavior of the P compounds has been shown to be a function of the NH_4', it is possible that the NH_4' is synergistic with Ca'' and Mg'' in certain functions of the body in which the lactic acid produced by muscular contraction also plays an important rôle.

That such a condition does exist is shown by the workers in Pawlow's laboratory. The work of this Russian school has recently been reviewed and translated by Prawdiz-Neminski (1924). From this extensive review, including the works of many Russian authors and investigators, it is concluded that the NH_4' in all forms of muscular and nervous activity unites with Mg'' and PO_4''' to form the triple phosphate and may be demonstrated in crystalline form in the tissues, thus removing these ions from solution and its ionic relationships. It is shown at the same time that excess of organic acids, notably lactic acid, redissolves the triple phosphate. The latter fact would seem of extreme importance in explaining why ammonium chloride alone is unable to maintain animals once in tetany free from tetany and death. The lactic acid already present would keep the NH_4' from uniting with Mg'' and PO_4''', thus making it necessary for more Ca'' to be in equilibrium with the excess PO_4''' ions. This same explanation may be attached to the fact that Mg'' treatment is effective only when the Ca'' is kept high. The Mg then acts as a pure depressant of all nervous elements, whereas, once the low Ca causes tetany to precipitate, the lactic acid so formed draws additional phosphate ions to the excess Mg'' ions, thus increasing the phosphates of the blood markedly.

Dogs treated with uranium nitrate. Eight thyroparathyroidectomized and 1 normal dog were treated with single injections of uranium nitrate. The symptomatology paralleled by the blood findings varied inversely with the age of the animal and directly with the dose administered.

Dogs can be rendered free from tetany and other symptoms of parathyroid deficiency for a period of 3 to 10 days before the severer symptoms of uranium poisoning appear as described by Swingle (1928). During this time the blood findings are similar to those found in the recovery period of the dogs treated with ammonium chloride except that the serum calcium does not show a tendency to rise. The inorganic P and T.A.S.P. reach

the same low level and are paralleled by the P/Ca ratios. The ratios however never reach the normal since the Ca does not increase.

The symptoms that appear after the 3 to 10 day (in one case 45 days) period of recovery are not identical to those found in parathyroid deficiency (as reported by Swingle and Wenner, 1926, 1928) but must be traced to the uremia or other injuries caused by the poisoning, since normal animals also show the same symptoms. When these symptoms of poisoning begin and as they progress to fatality (usually 5 to 10 days) both inorganic P and T.A.S.P. rise progressively and may be up to 7 and 3 times the normal, respectively, at the time of death.

This terminal increase in P paralleling the degree of uranium poisoning is attributed to excess tissue destruction caused by the uranium.

It is possible that here too the acidosis causing a decrease in the blood phosphates prevents tetany, but if the serum calcium content is an index of available Ca for functional activity, the uranium does not act by making the calcium more available to the tissues and blood, for the serum calcium does not exhibit a tendency to rise after uranium nitrate treatment.

General discussion. The facts in the literature together with this work have led to the opinion that the inorganic and total acid soluble phosphorus changes in the whole blood are not primarily etiological in the symptomatology and death of parathyroid deficient dogs. In the thyroparathyroidectomized dogs the inorganic P always increases but the total acid soluble phosphorus tends to decrease below the normal with the increase in time of post-operative onset of symptoms and the time of death after symptoms appear, and increase with the severity of the symptoms manifested. That is to say, as a rule, the earlier and severer the symptoms after operation and the severer the symptoms and the shorter the time leading to death after symptoms appear the higher is the rise in total acid soluble phosphorus of the whole blood, but if the symptoms are mild and delayed several days in their onset and if death is delayed several days after onset of symptoms, the total acid soluble phosphorus of the whole blood may be markedly below the normal at the time when symptoms appear or at the time of death.

A low blood inorganic and total acid soluble phosphorus (particularly the latter) is highly favorable to recovery. This is true when it occurs without treatment or when it occurs in the treatment by heparmone, calcium lactate, ammonium chloride, uranium nitrate, primarily in magnesium lactate, and parathormone when the calcium does not vary beyond ± 3 mgm. per cent relative to the normal.

The beneficial effects of prolonged treatment with magnesium lactate or morphine sulphate despite the secondary marked progressive increase in inorganic and total acid soluble phosphorus are attributed to the general and central depressant effects of the former and the central depressant effects of the latter and the depressant effect on respiration by both.

The rate of calcium change and not so much its absolute concentration is related to the onset of parathyroid deficient symptoms but seems to bear no relationship to the type and severity of symptoms initially manifested. That is to say, if the serum calcium drops 3 to 4 mgm. per cent in 24 hours, parathyroid deficient symptoms appear at the end of that period, as a rule, whereas if the calcium fall is spread over several days the decrease is usually as much as 5 to 6 mgm. per cent before symptoms appear, but the symptoms initially manifested, whether they be severe tetany, mild tetany or depression, do not depend on whether the fall in serum calcium is 3 to 4 mgm. per cent in 24 hours or 5 mgm. per cent in five days.

It follows therefore that the changes in concentration of neither the inorganic P nor the T.A.S.P. in the whole blood are definitely and constantly related to the concentration of the calcium in the serum either in extent or direction of change.

Still, when the symptoms were those of parathyroid deficiency or whenever death was directly due to the parathyroid deficiency and not to any toxic over-dosage in treatment, both P/Ca ratios were increased or decreased opposite to that of the calcium showing that the ratios changed largely as a result of the calcium change. Hence, when symptoms of parathyroid deficiency appeared or at the time of death (due to parathyroid deficiency) the ratios vary from 90 to 300 per cent (inorganic P/Ca) and from 60 to 150 per cent (T.A.S.P./Ca) above the normal.

But 2 dogs receiving no treatment showed in every way the same blood picture and clinical symptoms that ordinarily resulted in death (high inorganic P and T.A.S.P. and low serum calcium accompanied by mild tetany and severe depression), yet recovery occurred.

These facts would tend to show that the changes in serum calcium and in the acid soluble phosphorus compounds of the blood cannot be the only important factors that determine the symptomatology and death or recovery of parathyroid deficient dogs. Suggestions have been made that the ionic concentration of Mg'', NH_4' and particularly the K' as well as poisonous metabolites play a rôle in this whole syndrome.

Our knowledge concerning the various phosphorus compounds and the manner in which they are related to each other in the soft tissues and body fluids is at present hopelessly inadequate to draw any weighty conclusions. From all indications, the body can withstand a wide range of variation in both inorganic and total acid soluble phosphorus of the blood without any apparent effect on the organism as a whole. Greenwald (100) has increased the inorganic phosphate 20 times by phosphate injections without any very untoward effects. The conditions that cause a variation in these blood phosphorus compounds are so numerous even under physiological conditions that it seems presumptuous to estimate the functional and pathological importance of any given phosphorus compound

of the blood until the chemistry and the possible source of variation of all the phosphorus compounds of the body have been determined.

From the most recent work of Irving and Bastedo (1928) and that reviewed and extended by Sella (1928) it seems that the functional metabolism of muscle itself cannot be a direct source of inorganic phosphorus compounds of the blood. Moreover, Meyerhof and Lohmann (1928), Nachmansohn (1928), and Ferdmann and Feinschmidt (1928) followed independently by Fiske and Subbarow (1929) and Irving and Bastedo have established a mechanism of phosphorus metabolism within the muscle fiber that normally neither contributes to nor subtracts from the inorganic phosphorus compounds of the blood. In this intramuscular mechanism calcium and potassium are highly involved but their action has not been explained.

The whole mechanism hinges about a newly discovered compound called phosphocreatin—a phosphoric acid of methyl guanidine acetic acid $(OH)_2$ OP-NH-C-NH-N(CH_3–CH_2–COOH) which forms salts with K and calcium, both secondary and tertiary. The secondary salts but not the tertiary salts of calcium are easily given to hydrolysis under anaerobic and acid conditions, such as is brought about by muscular contraction. Under these same circumstances optimal conditions are created from the reformation of lactacidogen which involves the incorporation of phosphate. In this anaerobic and acid hydrolysis of phosphocreatin, creatin and calcium and potassium phosphate are probably formed. This whole process is reversible so that in the aerobic and more alkaline condition of muscular rest or between contractions the phosphocreatine is reformed. It is evident that if the easily hydrolizable K′ and secondary Ca″ phosphates are formed, the phosphates become readily available for the incorporation into lactacidogen, leaving an excess of well balanced Ca/K base for neutralization of the acids formed by muscular contraction. However, under abnormal conditions an excess formation of the tertiary calcium phosphate that is hardly hydrolizable in the muscle fiber will bring about an excess of the excitable K′ ions which will in turn draw excessively upon the Ca″ in the blood and cause increased diffusion of K′ into the blood.

It stands to reason that the parathyroids may function primarily in keeping this reversible reaction (phosphocreatin $\rightleftharpoons$ secondary K′ + Ca″ phosphates $\rightleftharpoons$ tertiary Ca″ phosphate) at an optimal level in the muscle and perhaps other tissues.

Since a disturbance in the Ca/K ratio has such a marked biological effect on the permeability and irritability it also stands to reason that metabolites otherwise ineffective may now have access to the cellular protoplasm perhaps too in increased amounts. Thus by a variation of cellular intoxication wide variations may be introduced into the syndrome of parathyroid deficiency.

In parathyroid deficiency the primary decrease in the serum calcium followed by a P retention would supply the demand of calcium and PO_4 in the tissue. The secondary retention of K and Na and their increase in the blood and tissues would be necessitated for the replacement of Ca'' which is being taken out of its ionic and chemical relationship with other compounds by being precipitated as the tertiary phosphate in the tissues (Greenwald, 1911).

It is thus obvious that it is very unlikely that the phosphates that are found in the muscle fiber and are directly related to its functional metabolism can be a direct source of acid soluble phosphorus increase in the blood. It is also obvious that the lower the phosphate concentration in the blood the less will be the extramuscular supply of phosphate to form the tertiary Ca phosphate in the muscle fiber and hence by the law of mass action more calcium will become available for its other functional relationships. Hence the beneficial effects of a low blood phosphate in parathyroid deficiency. Hence also, since the calcium supply in the body is so limited the PO_4 ion need not be in great excess to precipitate most of the Ca'' as the tertiary phosphate.

Since according to the above facts and postulations the phosphate is progressively precipitated in the tissues as the Ca phosphate in parathyroid deficiency, it may also explain the low phosphate in animals that do not show parathyroid deficient symptoms until several days after operation, particularly if the symptoms are mild.

It would seem then as Greenwald stated that the phosphate increase in the blood in parathyroid deficiency resulted from an excess destruction of tissue proteins and we would add that this and the terminal anuria found in both parathyroid deficiency and over-dosage are probably responsible for the increase not only in the inorganic P but also of the T.A.S.P. of the whole blood.

CONCLUSIONS

1. The average of 261 determinations on 71 normal dogs showed the following concentrations: Calcium 10.84 (milligrams per 100 cc. of serum), inorganic P. 4.44, total acid soluble P 26.65 (in milligrams per 100 cc. of whole blood), inorganic P/Ca ratio 0.41, and total acid soluble P/Ca ratio 2.42.

2. Although the phosphorus compounds, particularly of the skeletal muscles, may play a leading rôle in the symptomatology and death of parathyroid deficient dogs, the inorganic and total acid soluble phosphorus changes of the whole blood are not primarily etiological.

3. In thyroparathyroidectomized dogs the inorganic P always increases, but the total acid soluble P tends to increase with the severity of the symptoms manifested. However if the symptoms of parathyroid deficiency

are mild and delayed in onset for 3 or more days there is usually a fall to below the normal.

4. A low blood inorganic P and particularly a low total acid soluble P is highly favorable to recovery from parathyroid deficiency.

5. The beneficial effects of magnesium lactate and morphine sulphate despite a secondary progressive rise in inorganic P and total acid soluble P are attributed to their depressant action on the respiratory center and other nervous structures.

6. The rate of calcium fall, and not so much its absolute concentration in the serum, is related to the onset of parathyroid deficient symptoms but seems to bear no relationship to the type and severity of symptoms initially manifested.

7. As a rule both P/Ca ratios largely varied opposite to the calcium change and at the time of death or when symptoms of parathyroid deficiency appeared were increased by 90 to 300 per cent (inorganic P/Ca) and 60 to 150 per cent (T.A.S.P./Ca) above the normal.

8. Two out of 12 thyroparathyroidectomized dogs, none of which were treated, made a complete recovery though the blood picture and clinical symptoms were identical to those that died of parathyroid deficiency (high inorganic P and T.A.S.P. and low serum calcium accompanied by mild tetany and severe depression).

9. It is concluded that the behavior of the calcium and acid soluble phosphorus compounds in the blood of dogs suffering from parathyroid deficiency is largely due to a disturbance of phosphocreatin metabolism within the muscle fiber (and perhaps other tissues) which greatly involves the Ca/K ratio thereby affecting also the permeability and irritability of tissues whereby metabolites otherwise non-toxic occur in excess and gain access to the cellular protoplasm which results in the various clinical manifestations found in parathyroid deficiency.

10. The marked increase of the blood P compounds in early and severe parathyroid deficiency and parathormone over-dosage is attributed to the increased destruction of tissue proteins and a terminal retention.

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BLOOD PHOSPHORUS AND SERUM CALCIUM IN NORMAL AND PARATHYROIDECTOMIZED DOGS TREATED WITH PARATHORMONE

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The use of the active principle of the parathyroid glands, parathormone (Collip), since its discovery has largely turned to its employment clinically. The literature therefore consists almost entirely of its clinical application or its use in producing overdosage phenomena in animals. Parathormone overdosage both in normal and parathyroidectomized animals brings about a marked rise in inorganic phosphorus which is secondary to a marked calcium rise to above the normal value (Fisher and Larson, 1925). Collip (1926), Salvesen (1925) observed in parathyroidectomized dogs and cats a decrease in inorganic P (6 or 7 to 4 mgm. per 100 cc.) with a terminal rise as in the normals. Collip (1926) found in parathyroidectomized rabbits that with the calcium almost normal, injection of parathormone may bring about a rapid rise in inorganic P accompanying fatal tetany. They found increased excretion of Ca and P in the urine. These results have been affirmed by Greenwald and Gross (1925b, 1926) Grollman (1927) and others. It can thus be concluded that given a low serum calcium level, the effects of parathormone on the blood inorganic phosphorus is opposite to that caused by parathyroidectomy. No reports are available, as far as we have been able to find, on the behavior of the total acid soluble P of the blood in parathyroid deficient animals treated with parathormone.

Overdosage of parathormone brings about certain pathological changes that are especially prominent in the gastro-intestinal tract. Collip (1926) found a dark and hemorrhagic mucosa of the stomach as one of the most singular findings. Matthews and Austin (1927) specified that this change was confined to the fundus region. This was affirmed and its microscopical study extended by Heuper (1927).

The mode of action of this hormone is not understood. Its action has been attributed to its ability of changing water and mineral metabolism in which the calcium plays the major rôle. Just how this is brought about is today largely a matter of opinion and dispute and is outside of the scope of this research. Of interest is the fact that Collip (1926) was able to simulate the symptoms and the pathological findings recorded for parathormone

overdosage by careful injection of $CaCl_2$ and NaH_2PO_4. This was found only on one dog. Efforts in repeating the experiment met with failure.

The symptoms produced by parathormone overdosage are the same in normal and parathyroidectomized dogs. These symptoms together with the physical and chemical findings have been summarized by Collip (1926).

METHOD. After a brief control period one-half the dogs in this series were injected with massive (50 to 120 units) or a number of repeated small doses (10 to 20 units) of parathormone to test its effect on the normal animal. After return to normalcy these dogs together with the other dogs were completely thyroparathyroidectomized. Only a few dogs were allowed to go into tetany before treatment was started. The remaining dogs received treatment from the day following the operation. Both single daily massive doses and smaller doses at several hour intervals were injected, usually hypodermically. In some cases overdosage was produced. The blood analyses for serum calcium and whole blood inorganic and total acid soluble phorphorus[1] were made at various times before and after the injection of parathormone. Complete autopsies were done in nearly all cases to substantiate our clinical and chemical findings.

RESULTS. This report contains the data on 11 dogs. One of these dogs was not treated with parathormone until three months after operation or two months after showing no symptoms of parathyroid deficiency. This dog had recovered from typical depressions, mild tetany, and laryngeal stridor. The purpose was to note the effects during and after parathormone treatment on a parathyroid deficient dog not suffering from such deficiency. One other dog was discarded as abnormal after some days of preoperative treatment. Of the remaining 9 dogs, 2 were not treated until tetany had developed. The other 7 were treated from the day following the operation. Of these 9 dogs, 2 died of parathyroid deficiency on the 12th and 2nd days, respectively; 3 died of parathormone overdosage on the 4th, 9th, and 7th days, respectively; 2 died of undetermined cause on the 15th and 63rd days, respectively; and 2 dogs were killed—one on the 45th day because of severe ulcerations at the site of calcium injection, and the other on the 34th day by accidental puncture of a large coronary vessel in the process of obtaining a blood sample from the heart.

Dog treated 60 days after recovery from symptoms of parathyroid deficiency. This dog received no treatment whatsoever. It recovered from the symptoms of parathyroid deficiency in 30 days. Sixty days after this complete recovery the serum Ca, inorganic P and T. A. S. P. were, respectively, 7.52, 4.86 and 19.50 mgm. per cent. In the course of 20 hours 170 units of parathormone divided into 30 to 40 unit doses

[1] The serum Ca was determined by the Kramer and Tisdall (1921) method as modified by Clark and Collip (1925). The P determinations were made by the method described by Subbarow and Fiske (1925).

were injected at intervals of 3 to 5 hours. The Ca returned to normalcy where it remained for 4 days, then progressively decreased to below the

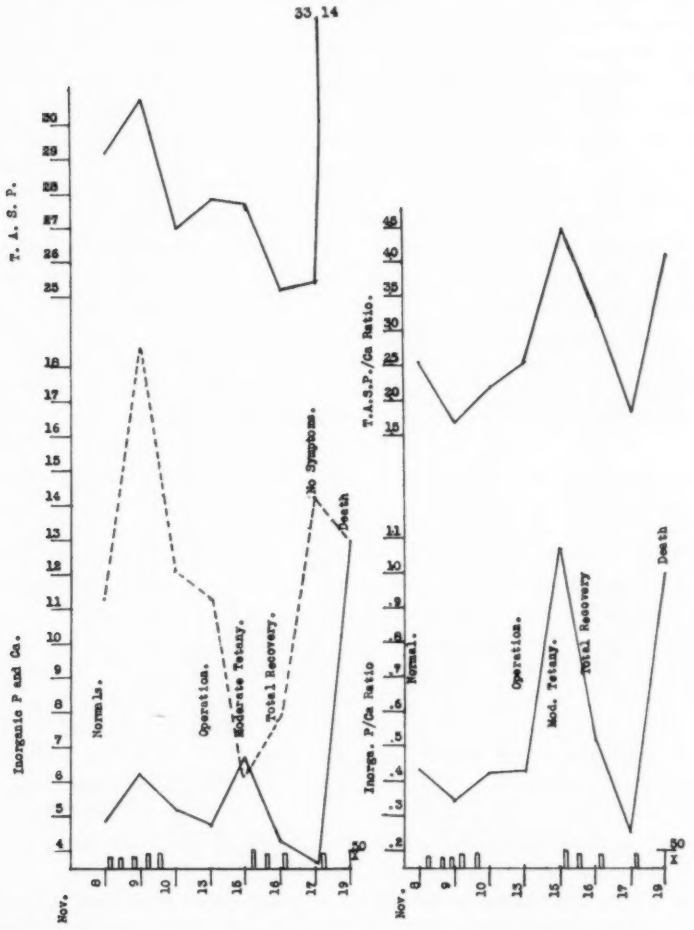

Fig. 1. Dog 121. Showing effects of parathormone before and after parathyrodectomy; also recovery from tetany when treatment is begun at the time of tetany. Death by overdosage. *X*, units of parathormone shown at the bottom of graph as vertical blocks. Ca in milligram per cent of serum. Inorganic P and total acid soluble P in milligram per cent of whole blood.

pre-injection level in 9 days. The inorganic P varied within the normal range, whereas the T. A. S. P., varying between 17 and 20 mgm. per cent

before parathormone treatment, was now (13 days later) 15.15 mgm. per cent. After this the animal became progressively worse, exhibiting more and more the symptoms of parathyroid deficiency until it died in severe

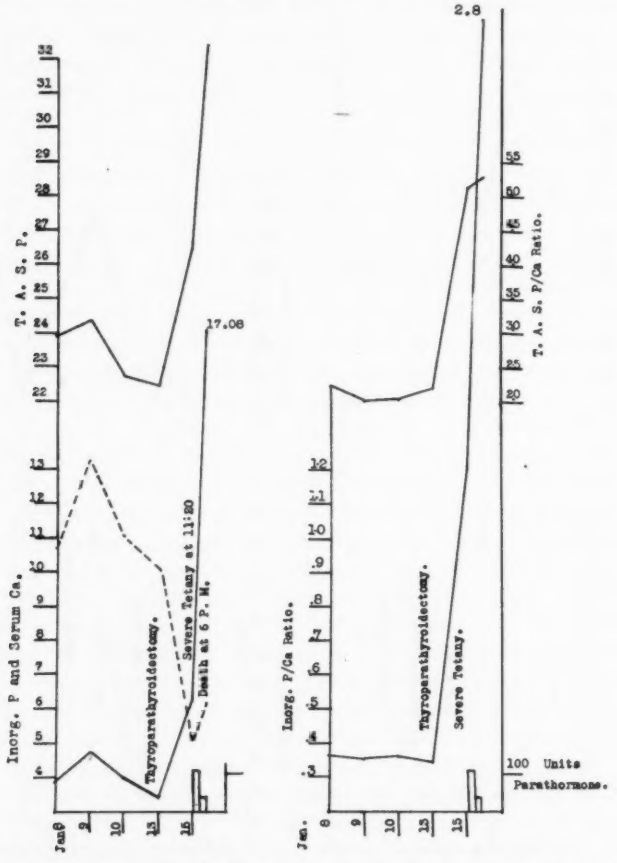

Fig. 2. Dog 125. Showing the effects of parathormone treatment in vertical blocks as abscissae in tetany when recovery does not follow. One hundred and twenty units parathormone at 11:20 and 40 units at 2:40 on the 15th. Dog died 3 hours later with parathyroid deficient symptoms. Ca in milligram per cent of serum. Inorganic and total acid soluble P in milligram per cent of whole blood.

convulsions 45 days after the parathormone treatment. Unfortunately no blood analyses are available on this dog during the last 15 days, but at this time both inorganic P (6.66 mgm. per cent) and T. A. S. P. (20.58) were on the incline.

No conclusions can be drawn from one animal. Since this dog was a female, the fatal convulsions may have been due to a period of heat (Dragstedt et al., 1924).

Dogs treated after tetany appeared. Of the 2 dogs treated after tetany appeared one which had been treated with parathormone before parathyroidectomy (dog 121, fig. 1) recovered completely, but died 3 days later of parathormone overdosage; the other (dog 125, fig. 2) showed alleviation of tetany but died 6½ hours after an initial injection of 120 units parathormone or 3 hours after an additional injection of 40 units parathormone. The difference in the behavior of the whole blood phosphorus compounds in these 2 animals is very striking. From the time of tetany to complete recovery, in dog 121 (fig. 1, Nov. 15 and 16) the changes in the concentration of serum Ca and whole blood inorganic P and T. A. S. P. were, respectively, 6.23 to 8.08; 6.66 to 4.03; and 27.76 to 24.76 mgm. per cent as compared to these respective concentrations in dog 125 (fig. 2, Nov. 15) at time of tetany and just after the animal had died 6½ hours later, 5.16 to 6.12; 6.25 to 17.08; and 26.50 to 32.45 mgm. per cent. The tetany, of course was more severe in the latter case than in the former before treatment was begun.

It seems from this that just as Collip (1926) observed in his parathyroidectomized rabbits that died despite the parathormone treatment, so it is also in dogs: an abrupt and sudden rise of the inorganic P of the whole blood (300 per cent in this case) with only a very slight change in the calcium content of this dog treated after severe tetany had begun. The T. A. S. P. rose only 6 mgm. per cent. This would probably have been much higher had the determination been made at least a half or one hour before death. We have found several times that the total acid soluble P at the instant of death is much decreased compared to its concentration a half or one hour preceding death (in one instance a drop from 40 to 29 mgm. per cent when drawn after death). These results would indicate that an abrupt and sudden rise in the inorganic P and T. A. S. P. without a commensurate rise in serum Ca after parathormone administration results in death, whereas if the parathormone results in recovery, the serum calcium is primarily increased accompanied by an inorganic P and T. A. S. P. fall. The latter two remain low unless the calcium is raised markedly above the normal (fig. 1, Nov. 17) when these values rise abruptly (Nov. 17 to 19—inorganic P 3.55 to 12.90 mgm. per cent; T. A. S. P. 25.38 to 53.14 mgm. per cent) and death ensues.

Dogs treated from the day following the operation. The results obtained on the 7 dogs treated from the day following the operation are shown by 3 representative dogs in the form of 2 graphs: a, dog 120 (fig. 3) shows the effects of high calcium before (Oct. 13) and after parathyroidectomy (Oct. 26) as a result of parathormone treatment followed by the effects

of a low calcium after parathormone treatment was withdrawn (Oct. 27 to Nov. 1); b, dog 129 (fig. 4) shows the effect of parathormone when the treatment is sufficient to keep the dog from any severe symptoms of parathyroid deficiency; c, dog 131 shows the effects of parathormone over-

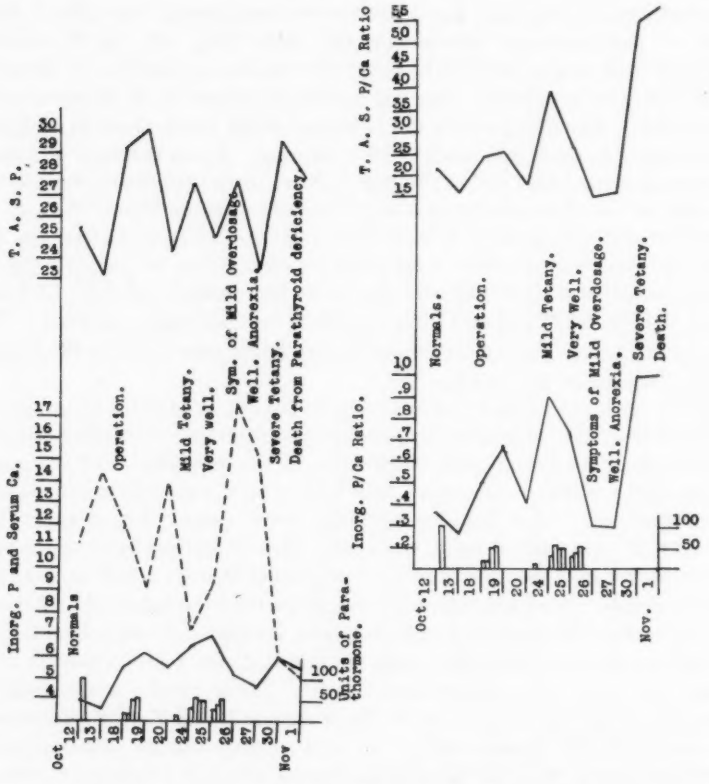

Fig. 3. Dog 120. Showing the effects of high Ca before and after thyroparathyroidectomy by parathormone treatment and followed by the effects of low Ca after treatment was withdrawn. *X*, 100 units parathormone 26 hours before operation. Otherwise the parathormone administered is shown at the bottom of the graph in vertical blocks in units and number of doses given daily. Ca in milligram per cent of serum. Inorganic and total acid soluble P in milligram per cent of whole blood.

dosage after parathyroidectomy. In this dog the treatment was withdrawn (no treatment after Jan. 9) before symptoms of overdosage appeared (Jan. 11). The clinical symptoms were those of parathormone overdosage up to the time of death (Jan. 13) despite the fact that the calcium was low.

The effect of parathormone on the whole blood inorganic and total acid soluble phosphorus can best be studied by a careful consideration of these graphs. It is shown in figure 3 (Oct. 12 to 13) that a moderate rise (3 mgm. per cent) of calcium in the normal dog is accompanied by a fall in the T. A. S. P. (2.3 mgm. per cent) and inorganic P (5 mgm. per cent) following the administration of a single massive dose (100 units). This

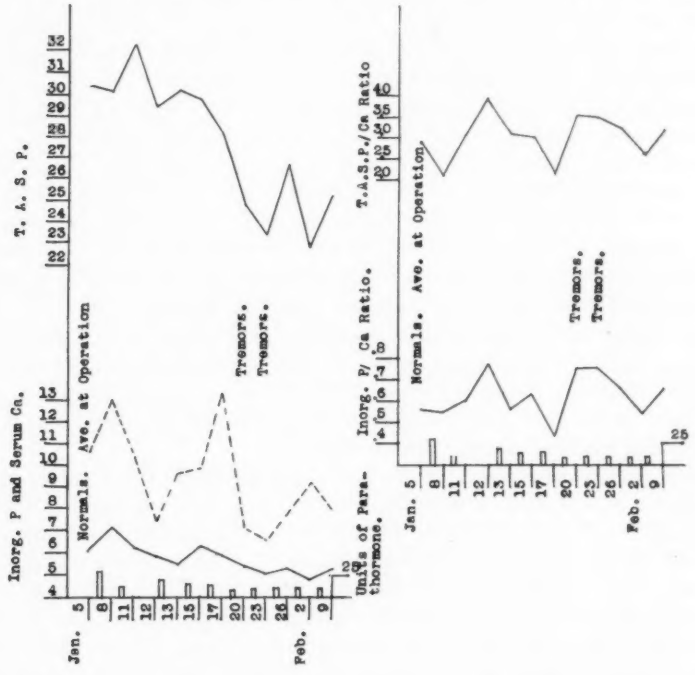

Fig 4. Dog 129. Thyroparathyroidectomized. Showing the effects of sufficient parathormone treatment (indicated in vertical blocks at the bottom of the graph in units and number of doses given daily) to keep the dog out of tetany. This dog was accidentally killed by bleeding from the heart. Ca in milligram per cent of serum. Inorganic and total acid soluble P in milligram per cent of whole blood.

whole relationship is reversed in the following days when the calcium returns to its normal level (Oct. 18); the T. A. S. P. (6 mgm. per cent) and inorganic P (2 mgm. per cent) rise. This primary fall in the blood phosphorus compounds did not take place if the massive dose was divided into 30 unit doses administered several hours apart. Both inorganic P and T. A. S. P. may then only exhibit a slight rise of 1 to 2 mgm. per cent

with a return to or slightly below the normal in the course of a few days when the calcium is again at the normal value (dog 121, fig. 1, Nov. 8 to 13; dog 123, Nov. 8 to 13; dog 125, fig. 2, Nov. 8 to 13).

After thyroparathyroidectomy, much the same relationship exists between the blood phosphorus compounds and the serum calcium under parathormone treatment. In all these cases (as shown by fig. 4) the general trend of both blood phosphorus compounds is progressively downward from day to day so long as the calcium does not vary markedly more than 2.5 to 3 mgm. per cent from the normal level. The inorganic P as is shown on January 6 and 15 (fig. 4) forms exceptions to this more often than does the T. A. S. P. of the whole blood. As a rule the T. A. S. P. tends to vary opposite to that of the serum calcium (fig. 3, Oct. 18 to 24); that is, as the calcium falls the T. A. S. P. rises and vice versa. But when this variation in the serum Ca is markedly below the tetany level (fig. 3, Oct. 30 to Nov. 1) or markedly above the normal 17.6 mgm. per cent on Oct. 26), the T. A. S. P. increases in both of these conditions. If this is preceded also by a marked rise in the inorganic P, (particularly in parathormone over-dosage) then both the inorganic P and T. A. S. P. rise progressively until death ensues, regardless of the behavior of the serum calcium (fig. 2). This graph indicates that in parathormone overdosage death is not only independent of the final serum calcium level but also that the inorganic P may be more than quadrupled and the T. A. S. P. more than doubled at the time of death.

It is obvious that the T. A. S. P./Ca and inorganic P/Ca ratios must exhibit a wide range of individual variations, since the serum calcium may range ± 3 mgm. from the normal before the phosphorus compounds are affected in relation to the serum calcium. This is shown on figure 4. These ratios exhibit no variations from those found in parathyroid deficiency without treatment when the calcium falls more than 3 or 4 mgm. per cent.[2] However, when parathyroid over-dosage occurs there may always be a marked rise in both of these ratios, since both phosphorus compounds increase progressively until death and the serum calcium decreases because of withdrawal of parathormone treatment.

Discussion and conclusions. Our results on the symptomatology as related to the serum calcium and whole blood inorganic phosphorus upon administration of parathormone to normal and parathyroid deficient animals affirm and extend those reviewed and established by Collip (1926).

Parathormone administration to normal and thyroparathyroidectomized dogs caused a progressive increase in the serum calcium, beginning 2 hours after the injections and reaching a maximum in 15 to 24 hours. After some hours it began to decrease and the course of decline was similar to

[2] A subsequent paper.

that of the rise. One dog injected until severe tetany occurred did not show this rise in the 6 hours before he died. In thyroparathyroidectomized dogs when by means of parathormone treatment the serum calcium was not allowed to vary more than ±2.5 to 3 mgm. per cent the inorganic P of the whole blood varied slightly from just above to 1 or 2 mgm. per cent below the normal, whereas the whole blood T. A. S. P. had a tendency to decrease at first markedly and then slowly and progressively. When these variations in the serum Ca were more or less beyond ±3 mgm. per cent from the normal, marked variations in the behavior of the inorganic P and T. A. S. P. took place. When the calcium variation was negative the results were the same as noted in untreated animals. When the serum calcium variation was on the positive side and did not exceed 5 or 6 mgm. per cent (from the normal) the T. A. S. P. change was opposite to that of the serum Ca; that is, when the serum calcium increased the total acid soluble P decreased and vice versa. When the serum calcium rose still higher accompanied by an abrupt increase in the whole blood inorganic P, then death ensued in 48 to 72 hours while the calcium fell progressively, whereupon the inorganic P and T. A. S. P. was as much as quadrupled and more than doubled, respectively, at the time of death. Death then occurred from overdosage symptoms even if the calcium level was at or below the tetany value. The abrupt and rapid increase of the inorganic P was the most constant finding when parathormone overdosage led to death, even in the dog that died 6 hours after tetany began and showing no increase in serum calcium from injection of parathormone. The increase in T. A. S. P. was just as constant but was not always so extensive and sometimes no higher at the time of death than when death occurred without treatment. At any rate, the inorganic P increase preceded the T. A. S. P. increase in those dogs that died of overdosage.

In consequence to the above the inorganic P/Ca and T. A. S. P./Ca ratios vary markedly when the Ca does not vary beyond ±3 mgm. per cent from the normal, but beyond this ±3 mgm. per cent variation these ratios are both increased and at the time of death due to overdosage the inorganic P/Ca ratio may be increased by 3 to 7 times and the T. A. S. P./Ca by 0.5 to 3 times.

SUMMARY

1. Our results on the symptomatology as related to the serum calcium and whole blood inorganic phosphorus upon the administration of parathormone to normal and parathyroidectomized animals affirm and extend those reviewed and established by Collip (1926).

2. The relation of the serum calcium to the inorganic and total acid soluble phosphorus in parathyroid deficiency and parathormone overdosage is discussed. It is found that parathormone reduces the inorganic and

total acid soluble phosphorus of the whole blood until the calcium rises markedly above the normal. The inorganic phosphorus then rises rapidly usually followed by a marked rise in the total acid soluble phosphorus until death ensues.

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THE INFLUENCE OF LIVER EXTRACT ON DOGS SUFFERING FROM PARATHYROID DEFICIENCY

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During the last few years a number of articles (Major, 1925, 1926) have appeared setting forth the action of certain liver extracts which produce a lowering of blood pressure. This depressor action is said to be most pronounced in patients suffering from hypertension or in cases of experimental hypertension produced by intravenous injections of guanidine or methyl guanidine salts (Major, Stoland and Buikstra, 1926). Quite recently Miller and Martinez (1927, 1929) have successfully treated toxemias of pregnancy with liver extract (heparmone).[1]

If the liver extract has the ability to neutralize toxic substances produced under abnormal conditions, and especially if it neutralizes the action of such substances as guanidine and methyl guanidine, it seemed to us possible that it might counteract the toxemia produced in parathyroid deficiency. We grant that the theory that guanidine or methyl guanidine is responsible for tetany in animals suffering from removal of the parathyroids has met with a considerable amount of opposition, nevertheless the possibility remains that the toxemia may be an important factor in producing the symptoms following parathyroid removal.

In order to determine whether the parathyroid tetany could be counteracted by the liver extract a series of experiments was undertaken in which thyroparathyroidectomized dogs were treated with varying doses of the liver extract on the development of tetany. The clinical symptoms were followed in these animals and the blood calcium determined.

The results here reported include experiments on twenty-seven dogs, four of which were used as controls, in that they were not treated with liver extract.

No attempt was made to prolong the life of the animals treated with liver extract by calcium therapy.

A group of fourteen dogs developed distinct parathyroid tetany following operation. Nine of this group recovered from the tetany following the injection of liver extract and lived from six to twenty days, or an average

[1] The liver extract used in the experiments reported in this paper was generously supplied by Eli Lilly & Company.

of ten days. The liver extract treatment was discontinued before death in fully one half of these cases. It is possible that the length of life might have been further prolonged had the treatment been continued. Three of this group did not show any improvement and died from the first attack of tetany two days, two days and one day after the operation respectively. Two animals were not treated with liver extract on the development of tetany and both died three days after the operation, each having had more than one attack of tetany.

These results show clearly that when the conditions that are responsible for tetany are to a certain degree counteracted by the liver extract, the survival period is prolonged. If, as happened in these animals, the injection of liver extract does not neutralize the factors that produce tetany, the animals fail to survive the first attack.

The liver extract was administered in different amounts during various periods of time. In our earlier experiments we used small doses (from 5 to 10 cc.), the total per day never reaching above 50 cc. The injections were variously distributed during the observation period, which was usually from five to twelve hours. Later some dogs were systematically injected with a total of from 30 to 60 cc., divided into 5, 10, 15 or 20 cc. doses, the time between the injections being five, ten, fifteen, twenty or thirty minutes until the full amount was injected. Lastly we have resorted to the large massive doses of from 40 to 75 cc. at the beginning of each observation period. In all cases the effect on the blood calcium is essentially the same. Clinically, the immediate visceral symptoms to be described later are not evident with injections of small doses of from 5 to 20 cc., although the momentary increase in tetany or depression when already present usually occurs even with so small a dose as from 5 to 10 cc. The course to recovery is usually more delayed (not always) with the smaller doses when used in depression and tetany dogs, but the period of tetany is not strikingly lengthened.

Clinical symptoms following liver extract injection. As is well known, removal of the parathyroid leads to two types of symptoms; namely, 1, the type in which violent tetany is developed, and 2, the type in which the animal becomes depressed. Our series includes both types. When liver extract was administered to animals suffering from tetany or depression the symptoms became more severe during a period of from two to five minutes following the intravenous injections of 25 to 40 cc. or more. This is accompanied by certain visceral reactions consisting of marked salivation, borborygmus, passing of flatus, forced micturition and violent intestinal movements followed by defecation. Vomiting movements usually occur; respiration becomes deeper and slower, and the heart beat becomes so faint that it can hardly be felt. In fifteen minutes or sooner the heart beat becomes abnormally pronounced. The animals recover from these

visceral symptoms in from three to ten minutes after the injection of liver extract. Complete recovery from tetany occurs from fifteen minutes to two hours after the injection except in the three cases already referred to, in which there was failure to recover. The symptoms described indicate that the liver extract has caused a marked stimulation of the parasympathetic innervation. The marked fall in blood pressure following the injection occurs during the period of the visceral symptoms described. Whether the fall in blood pressure is responsible for the visceral symptoms or merely accompanies them is not clear. In every case in which blood pressure was recorded in animals suffering from tetany a sudden fall occurred and the pressure returned to nearly normal within five minutes after the injection (fig. 1).

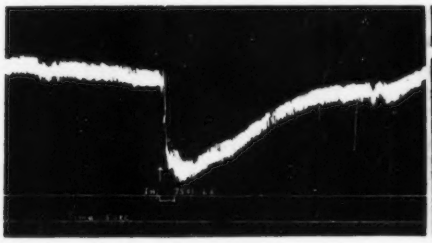

Fig. 1

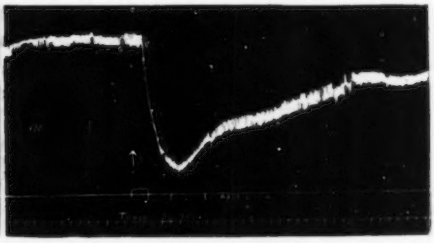

Fig. 2

Fig. 1. Blood pressure record of dog weighing 12.4 kgm., suffering from parathyroid tetany, showing the effect of intravenous injection of 75 cc. of liver extract.

Fig. 2. Blood pressure record of normal dog weighing 6.8 kgm., showing the effect of intravenous injection of 30 cc. of liver extract.

The coagulation time of the blood of these animals was distinctly shortened following the administration of the liver extract, as shown in the accompanying table.

When liver extract is administered to normal animals there is as pronounced a fall in blood pressure as in the case of animals suffering from parathyroid deficiency (fig. 2) but the other visceral reactions do not occur. This would indicate that the fall in blood pressure is not the cause of the visceral reactions. The animals suffering from parathyroid deficiency seem to have become hypersensitive to the liver substance, as shown by the visceral reactions. On repeated injection of the liver extract, the visceral symptoms become less pronounced and more delayed, and finally are not perceptible on general observation.

A group of five dogs that were treated with liver extract daily after the removal of the parathyroids showed about the same course in so far that the visceral reactions occurred following each injection. Two

never developed parathyroid deficiency symptoms but died within four days. The other three developed mild tetany which was counteracted by liver extract treatment to the same degree as in the animals treated following the development of tetany. These results indicate that whatever beneficial reactions are induced by the liver extract treatment depend on the ability of the liver extract to counteract the tetany or depression rather than to protect the animals against the development of these symptoms.

Serum calcium levels following liver extract treatment. In all our animals the serum calcium level was determined by the Kramer and Tisdall method as modified by Collip and Clark (1925). All the animals exhibited the usual drop in serum calcium level following parathyroid removal (except one bulldog). In all the animals which improved on the adminis-

TABLE 1

Effect of liver extract on coagulation time of blood

Blood coagulation time in three dogs suffering from parathyroid tetany and one normal before and after injection of liver extract. Ether anesthesia.

DOG	COAGULATION TIME BEFORE LIVER EXTRACT INJECTION		COAGULATION TIME FROM ONE TO TWO MINUTES AFTER INJECTION OF LIVER EXTRACT		COAGULATION TIME FROM FIVE TO EIGHTEEN MINUTES AFTER INJECTION OF LIVER EXTRACT	
	minutes	*seconds*	*minutes*	*seconds*	*minutes*	*seconds*
Dog 74 in tetany	6	30	1	30	3	
					1	
					4	
Dog 75 in tetany	5		2	30	7	
Dog 76 in tetany	5		2		2	
Dog 77 normal	6			50	2	30
					3	
					1	

tration of liver extract the serum calcium level fell from 0.1 to 1 mgm. per 100 cc. of blood from one to three hours following the injection with a return to the pre-injection level from two to five hours after the injection (fig. 3). In the three animals that failed to improve from the liver extract treatment the serum calcium increased from 1 to 2 mgm. per 100 cc. blood (fig. 4).

In the three dogs treated with liver extract before thyroparathyroidectomy, two days' treatment with from 40 to 50 cc. of liver extract brought a fall in calcium of from 1 to 5 mgm. (*A* and *B*, figs. 3 and 4) Of these three, later thyroparathyroidectomized, one developed severe tetany and was not benefited by the liver extract (*D* in fig. 4); one developed tetany and was benefited by the liver extract, living nine days, and the other developed depression and died in twenty days under treatment with liver

extract, from which it showed improvement (*D*, *E*, and *F* in fig. 3). After thyroparathyroidectomy the calcium level followed the same course of the respective group in which each fell as described above.

The dogs with acute tetany that were not treated by the liver extract showed a gradual decline in the blood calcium until death, while the depression dog which recovered showed a gradual decline until the ninth day

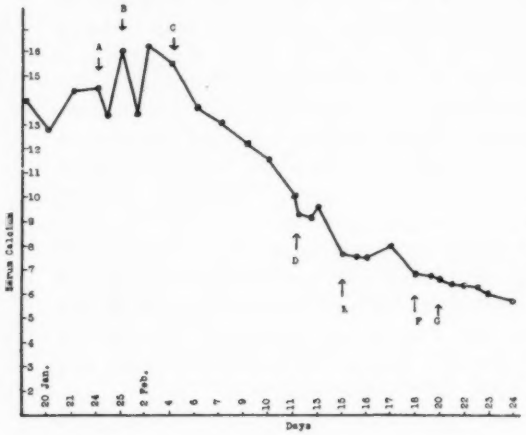

Fig. 3

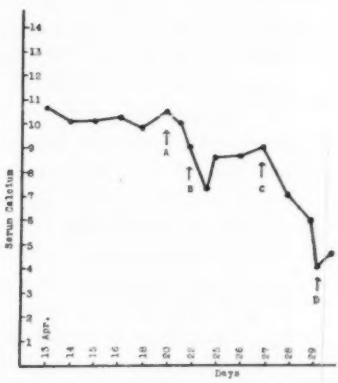

Fig. 4

Fig. 3. Calcium in milligrams per hundred cubic centimeters of serum in dog 60, a female, weighing 10 kgm., treated with liver extract before and after thyroparathyroidectomy. The dog was benefited by the extract. *A*, 40 cc. of liver extract, no symptoms; *B*, 40 cc. of liver extract, no symptoms; *C*, thyroparathyroidectomy; *D*, depression—65 cc. of liver extract, followed by recovery; *E*, depression and mild tetany—90 cc. of liver extract, followed by recovery; *F*, depression and mild tetany—70 cc. of liver extract, followed by recovery; *G*, tetany—55 cc. of liver extract, followed by recovery.

Fig. 4. Calcium in milligrams per hundred cubic centimeters of serum in dog 54, a male, weighing 10 kgm., treated with liver extract before and after thyroparathyroidectomy, and not benefited by liver extract injection on development of tetany: *A*, 30 cc. of liver extract, no symptoms; *B*, 50 cc. of liver extract, some depression; *C*, thyroparathyroidectomy; *D*, tetany—30 cc. of liver extract, no recovery.

after the operation, after which the calcium level remained about 5 mgm. per 100 cc. of blood even following recovery twenty days after the operation, when the last determination was made.

These results indicate that whatever benefits are obtained from liver extract treatment are not caused by its effect on the blood calcium, unless the slight fall indicates an increased utilization of calcium. The results further support the contention that factors other than the low calcium are responsible to a certain extent for symptoms of parathyroid deficiency.

Inorganic and total acid soluble phosphorus of the whole blood following liver extract treatment. Since no characteristic line of symptoms can be attached to the inorganic phosphorus, the blood content of this element being so variable under various conditions, we attach no particular significance to it in these investigations. All of the dogs benefited by the liver extract, and one not benefited by the liver extract showed a tendency initially to decrease the inorganic P sometimes to one-half its normal level, and then follows in $1\frac{1}{2}$ to 5 hours a compensatory rise which may be up to twice the normal. This change is most marked where the dosage is divided and injected at intervals of 5 to 15 minutes at the beginning of the observation period. The other 2 dogs not benefited by the liver extract only increased the already rising inorganic phosphorus until the ratio to the calcium was approximately 1. But a dog in tetany may die with an inorganic phosphorus level as low as 1.85 or as high as 10.00 mgm. per 100 cc. whole blood, and in one case the inorganic phosphorus was 19.4 immediately after death, having been more than twice the calcium concentration during the entire day. The untreated dogs showed a gradual rise of 1 to 5 mgm. of inorganic P in the acute tetany dogs, and a gradual post-operative rise in the depression dog until the ratio to the calcium (P/Ca) was less than 1, where it still was on recovery 20 days after the operation. The untreated dogs are discussed more fully in another paper.

All the tetany and depression dogs benefited by the liver extract, and all of the dogs treated daily after operation showed an initial increase of 1 to 6 mgm. of total acid soluble phosphorus per 100 cc. of whole blood wherever the determinations were made 5 and 15 minutes following the injection after which a progressive fall set in, at times exceeding 5 mgm. below the pre-injection level. If at any time during successive days of treatment this compensatory fall in the total acid soluble phosphorus did not take place, the animal soon died. In all of these dogs, after 2 to 6 periods of injection when the visceral symptoms were faint and delayed or no longer evident, the total acid soluble phosphorus did not show this initial rise, but progressively decreased from the time of injection. The acute tetany dogs that were not benefited by the liver extract showed a more rapid rise of the already increasing phosphorus, and before death in 1 to $2\frac{1}{2}$ hours reached a level of 32 to 40 mgm. of total acid soluble phosphorus for 100 cc. of whole blood. We are quite certain that the total acid soluble phosphorus initially rises in the first 2 hours from 1 to 3 mgm. and then gradually returns to the pre-injection level. The acute tetany dogs not treated by the liver extract showed a progressive rise in the total acid soluble phosphorus reaching from 5 to 15 mgm. per cent above the normal when death occurred, while the untreated depression dog showed a progressive decrease until symptoms appeared when it gradually rose with

the severity of symptoms to 8 mgm. above the normal in the course of 5 days and then gradually came back to normal in 4 or 5 more days, when symptoms again disappeared and the dog ate a full meal without aid of stomach tube.

There is no constant tetany level for the total acid soluble phosphorus. Neither is there any relation between the post-operative line of symptoms and the normal level of the total acid soluble phosphorus any given dog may have maintained before operation, such as a high normal indicating severity of symptoms after operation or vice versa. One may rather say that the total acid soluble phosphorus is kept below normal when the calcium is low where symptoms are alleviated or absent.

All phosphorus determinations were made by the method of Subbarow and Fiske (1925).

SUMMARY AND CONCLUSIONS

1. Liver extract (heparmone) when injected intravenously into dogs suffering from parathyroid tetany or depression produces marked visceral reactions.

2. The visceral reactions are accompanied by an increase in the severity of the tetany or, if depression is present, by induced tetany of short duration.

3. The visceral reactions are followed by a recovery from tetany. In three dogs which failed to improve after treatment with liver extract, no noticeable visceral reactions developed.

4. The serum calcium level is slightly lowered in animals suffering from parathyroid tetany and in normal animals on the administration of liver extract. In three animals not benefited by liver extract the calcium level rose from 1 to 2 mgm.

5. A marked fall in blood pressure follows the injection of liver extract and occurs simultaneously with the visceral reactions as judged from the time the two occur following injection.

6. The coagulation time is distinctly reduced during the period of low blood pressure following the injection of liver extract.

7. The recovery period of thyroparathyroidectomized animals is not markedly prolonged by the administration of liver extract.

While our results indicate that liver extract may counteract the toxemia of parathyroid tetany, it is not nearly as effective an agent as calcium and other substances. We would suggest extreme caution in the administration of large doses of liver extract in patients suffering from any type of toxemia.

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THE ACTION POTENTIAL IN FIBERS OF SLOW CONDUCTION IN SPINAL ROOTS AND SOMATIC NERVES

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The conducted action potential of mixed somatic nerve when recorded, after amplification, by the cathode ray oscillograph exhibits waves, the more constant and prominent of which, for the sake of convenience, have been designated *alpha*, *beta*, *gamma* and *delta*[1] in the order of their appearance (Erlanger, Gasser and Bishop, 1924). These waves have always been confluent, with the exception of the one in the saphenous nerve of the dog, called *delta*, which, at sufficient distances of conduction, is separated from the waves preceding it by a stretch, labelled *gamma* (Erlanger, 1927), lying almost, if not quite, at the level of zero potential. With the high amplification now used it turns out that an upward convexity of this "*gamma*" portion of the saphenous record, when present at all, is insignificant in comparison with the other elevations of the action potential; evidently it was a mistake to dignify it with a special designation.[2] Recently a wave spaced similarly to the "*delta*" in the saphenous has been found in other warm-blooded somatic nerves and in cold-blooded nerves; in addition, waves even later still have been found in the action potentials of both warm- and cold-blooded nerve, somatic (Gasser and Erlanger, 1929) and autonomic.[3] The present paper deals primarily with the waves of slow conduction as seen in somatic nerves and spinal roots.

The waves with which we are here chiefly concerned failed until recently to appear in our records for one or more of three reasons, namely: *a*,

[1] This *delta* wave was observed in our original records only occasionally; and now, in records made at an amplification of 100,000, we again find it to be quite inconstantly present. Under the circumstances it seems best for the present to disregard this wave, and consequently, in what follows, we make no attempt to account for a *delta* potential wave.

[2] Probably the notch on the down stroke of the wave designated *beta* in the published records (Erlanger, 1927) marks the separation, in the dog's saphenous action potential, between the waves homologous with the *beta* and *gamma* waves of cold-blooded action potentials.

[3] A parallel investigation of nerves of the involuntary nervous system has been carried out by Heinbecker and Bishop in this laboratory (1929).

they were too low in amplitude to be discernible; *b*, the fibers contributing to their action potentials have such high thresholds that they were not stimulated by the shocks originally employed; and *c*, they are propagated so slowly that they fall outside the limits of the records as they were then made.

a. The modification of our amplifier, whereby, merely through the turning of a switch, a fourth panel can quickly be added to the three of which it originally consisted, thus making conveniently possible 100,000 fold amplification as compared with the previous limit of 6,000 to 8,000, has brought to visible amplitude the unsuspected small, slowly propagated action potential waves, some of which may have less than a hundredth the amplitude of the main deflection.

b. For the study of the initial processes of the action potential it is very desirable to use as stimuli electrical currents of very short duration. The shortest induction shock conveniently available is that supplied by the Porter inductorium with core removed; and this, therefore, was employed for the most part as the source of the stimuli heretofore used. It turns out, however, that the less irritable fibers with which the present paper is concerned may require for stimulation the strongest shocks obtainable from the coreless coil with three volts in the primary circuit. To be sure of obtaining the necessary range of stimulation strengths, it is advisable to use the cored coil.

A few words are necessary here regarding the stimuli employed. The Porter coil used throughout the research was modified by extending the ways for the secondary so that the entire range of stimulation strength could be obtained without the necessity of turning the secondary coil. The primary current was supplied usually by two or three dry cells in series, and there was a resistance, usually of about 1.5ω, in series with the primary, and a 30ω shunt across the primary terminals so as to minimize an artefact that tends to distort the record. The coils begin to overlap at 6.5 cm. In order to have some notion of the relative strength of the stimuli employed under the conditions of an experiment, the heights of the shocks were measured directly on the cathode ray oscillograph at different coil positions with the primary voltage and primary and secondary resistances most commonly obtaining. From the data thus obtained a curve has been plotted of the shock height (voltage) against coil position. Though, from such a curve the absolute height of the shocks under different conditions cannot be ascertained, it is possible, when the coil position for any one response is known, to ascertain roughly the strength relative to this of the shock used for any other response from the same preparation.

With high amplification the escape from these strong shocks becomes a prominent feature and may seriously distort the base line on which the action potential is written. By using Bishop's balancing device (1926) this escape usually can be almost completely balanced out. Occasionally, however, some of it remains as a curve of unascertainable form upon which the record is written as a base line. In this connection reference should be made also to the possibility (reported at the Edinburgh Congress (1923), but not published) of there being a long lasting residual

potential upon which as a base line the successive axon action potentials would be recorded. Any such residuum and any shock distortion render impossible the exact determination of the position of the base line. In measuring amplitudes and areas of deflections, therefore, we have usually taken into consideration only transient elevations, not elevation above the zero level.

c. Our timing condenser (1 mf.) charging through 2,000 ohms and producing what we call "a 2,000 ohm line" moves the spot across the screen of the oscillograph at such a rate as to give about the most convenient intervals per *sigma* and total duration of deflection for the analysis of the parts of the action potential heretofore described; and in our first studies this was the speed of time line usually employed. Exact analysis is possible through about 5 to 6 σ. The wave in the saphenous nerve of the dog, heretofore designated *delta* (now *B*) (Erlanger, 1927), appears, in the lengths of nerve usually employed, something like 9 σ from start of the record; it was found in a 5,000 ω line. Lines lasting effectively longer than 9 σ were rarely employed. In cold-blooded nerve the wave which, as will be seen, corresponds with this late wave in the saphenous, requires for its demonstration a record lasting through 30 σ or more; it is obtained by a resistance of 20,000 to 30,000 ω. To permit the slowest waves to appear upon the record it has been found that the recording time may have to last 300σ or even longer, and for this a resistance of 150,000 to 200,000 ω in the condenser circuit is needed.

With these slow rates of movement of the spot a single deflection suffices for a legible record, on ordinary process film, even. To obtain such a record the rate of revolution of our timing apparatus is reduced so that the nerve is stimulated at the rate of about 10 shocks per minute and the film is held against the face of the tube so as to be exposed to just one of the transits of the fluorescent spot. Legible single deflections at higher speed can be obtained by the method described by Gasser, using duplitized film (1928, a and b).

THE SLOWER WAVES IN SOMATIC NERVES: DESCRIPTION OF TYPICAL RECORDS. Figure 1, *a* shows the action potential in the sciatic nerve of the bullfrog, after 91 mm. of conduction, on a 5,000 ω line as we usually have recorded it, excepting that the shock is stronger. The terminal phase of the shock escape extends under the action potential, but the deformation it produces is inconsiderable. Excepting this slight deformation, the picture, with its *alpha*, *beta* and *gamma* waves, is quite typical of the conducted action potentials previously published. The same action potential with full four-panel amplification is reproduced as *b*. Homologous features in records *a* and *b*, as well as in all subsequent records, can be identified by the labelling. The increase in amplification is given by the ratio of the heights of the two *gamma* waves; it is about fifteen-fold. The *alpha* and *beta* waves and the down stroke of the shock escape far

exceed the limits of the record, and the spot moves so rapidly here that its impression on the film is almost illegible.

By increasing the resistance in the condenser (spot-moving) circuit from 5,000 to 19,000 ω, the line now was prolonged so as to last effectively about 50 σ and, without changing any other of the conditions, records *c*

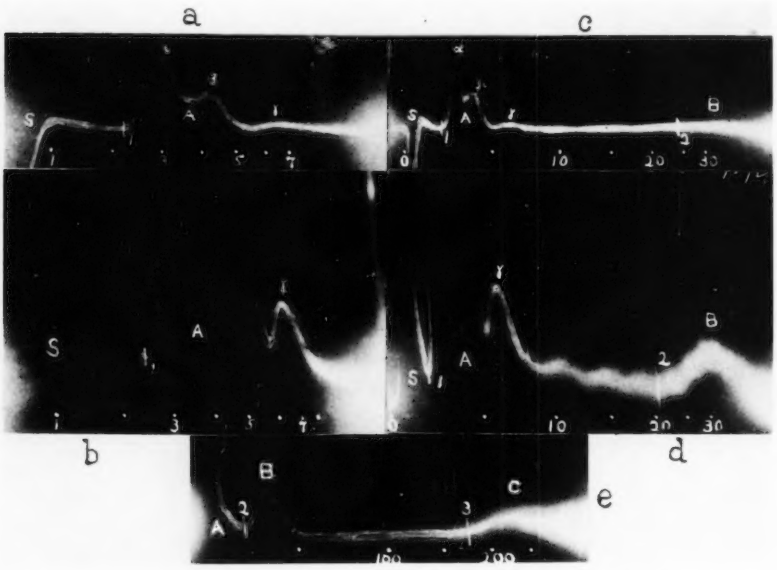

Fig. 1. Records from bullfrog sciatic, 1/31/29 (retouched). Conduction distance 9.1 cm. Temp. 21°C. In this and in all other records *S* = shock artefact; *1* = start of *A* elevation, *2* = start of *B* elevation, *3* = start of *C* elevation. × 3/5 approx. In all figures, unless otherwise specified, the time in σ is indicated by dots below each record, zero (sometimes ahead of record) being the start of the induction shock.

a, 3 panels, 5,000ω, 1 mf. (1 mf. in all other records unless other capacity is mentioned). Stimulus maximum for *B*, coil at 7.5 cm.

b, 4 panels, otherwise same as *a*.

c, 3 panels, 19,000ω, otherwise same as *a*.

d, 4 panels, otherwise same as *c*.

e, 4 panels, 149,000ω. Stimulus maximum for *C*, coil at 3.9 cm. Conduction rates of *A*, *B* and *C* are 42.0, 4.36 and 0.56 m.p.s., respectively.

and *d* were made with three- and four-panel amplification, respectively. The shock escape and the *alpha-beta-gamma* group of waves now are further forward and closer together spatially, but not temporally. Toward the end of the line in *c* close examination reveals (at *2*) a slight upward undulation (*B*); in *d* this is magnified into a wave 9 to 10 mm. high, becoming a

prominent feature of the record. At this amplification the estimated height of the *A* elevation is about 174 mm. The intervals between the shock and the start, crest and end of the *B* elevation are 20.89, 30.06 and 53.53 σ, respectively. Between the end of *gamma* and the beginning of *B* the potential is low and steadily declining, but how much of this is due to newly arriving axon potentials and how much to displacement and deformation of the base line by shock escape and residual potentials it is impossible to say.

For *a*, *b*, *c* and *d* of figure 1, the strength of the stimulus was so chosen as to elicit a just maximal *B* wave; the coil position was 7.5, that is to say, the secondary was 7.5 cm. from home. The threshold position of the coil for the *alpha* wave was 25.5 cm. and for the *B* wave 11.8 cm.

Finally, the movement of the spot across the face of the tube was slowed still further by raising the resistance in the condenser circuit to 149,000 ω, thereby obtaining a line lasting usefully through something over 300 σ, and the strength of the shock was increased by moving the secondary coil to 3.9 cm. The shock escape now (fig. 1, *e*) is at the beginning of the line and indistinguishably fused with *A;* the *alpha-beta-gamma* (*A*) complex is completed in the first 10 mm. of the record, but is so quick that only the end of it becomes visible, and it is succeeded by the *B* wave. Following this, and lasting about 0.1 second, there is a long, practically horizontal, stretch of record a trifle below the base line. This depression of the base line is attributed partly to the shock artefact and partly to the diphasic artefact. A strictly monophasic action potential is impossible of attainment (Bishop, Erlanger and Gasser, 1926) and the slower the propagation of an action potential wave the shorter must be the distance between the leads in order to mask diphasic effects. For this reason, the *B* wave, and especially the *C* wave, are apt to exhibit diphasicity even when *A* appears to be monophasic. These displacements render impossible the exact determination of the intermediate potentials. In this case, we may assume, however, without introducing any great error, that the *B*- to -*C* potentials, were it not for these artefacts, would have recorded a trifle above the base line. The record is terminated by a low wave labelled *C*. The start, crest and end of this wave follow the shock 163.8, 203.3 and 314.0 σ, respectively. The shock strength used was just maximal for this *C* wave. The threshold position of the secondary coil for the *C* wave was about 7.6 cm.

Some conception of relative stimulation strengths at the coil positions given above is obtainable by reference to the curve we have plotted of coil separation against shock amplitude. At separations of 25.5, 11.8, 7.5 and 3.9 cm. the relative shock heights are 1, 2.8, 16.7 and 152, respectively.

The entire picture in one record, but of another, and shorter, bullfrog sciatic, is seen in figure 2. The stimulus was a single shock that was

maximal for the C wave, the record being made at full four-panel amplification by a single transit of the spot across the screen at a rate determined by 200,000 ω in the timing circuit. The retention of the A and B potentials deforms the base line. The arbitrarily assumed zero line has been drawn into the figure in a form that would give to this record about the configuration of other, less deformed, records. The action potential obviously is made up of three main elevations, A, B and C, of which A together with the shock artefact can be recognized merely as an interruption in the deflection; the A amplitude far exceeded the limits of the film. Some idea of the voltages of the elevations can be obtained by taking the

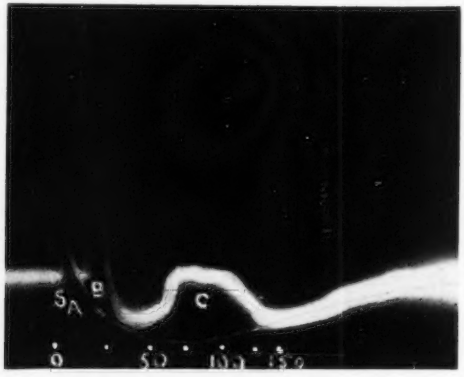

Fig. 2. Record (retouched) of a single deflection of the spot with full 4-panel amplification and 200,000ω in condenser circuit, bullfrog sciatic, 11/13/28. Conduction distance 4.0 cm. Temp. 23°C. Stimulus maximum for C, coil at 1.5 cm. Natural size.

The quick phase of the shock artefact and the A elevation are merged. Their height exceeds many times the limits of the film. Conduction rates of B and C are 4.0 and 0.46 m.p.s. Natural size.

sensitivity of the tube in round numbers as 10 volts equals 1 cm. at three-panel amplification (6,000 times). Then the A, B and C deflections in figure 1 subtend 23.2, 1.3 and 0.5 millivolts, respectively.

Figure 3 shows the action potential of the bullfrog sciatic (data derived from the records of fig. 1) drawn into a linear coördinate system from the logarithmic time coördinates of the records by joining with straight lines the points representing the starts, crests and ends and notches of the several waves. The height of the connection between A and B is taken directly from the record, the stretch connecting B with C has arbitrarily been placed a trifle above zero level. In the figure thus obtained the A elevation, made up of the *alpha*, *beta* and *gamma* waves, is seen to form a

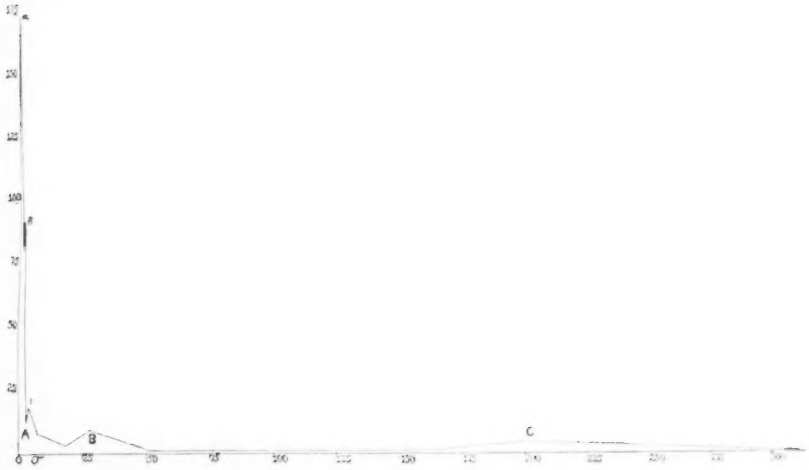

Fig. 3. The action potential of figure 1 transferred to linear coördinates by joining the points indicating the starts, crests, ends and notches of the *A*, *B* and *C* waves, the start of *A* resting on zero. Abscissae, time in σ; ordinates, amplitude in millimeters.

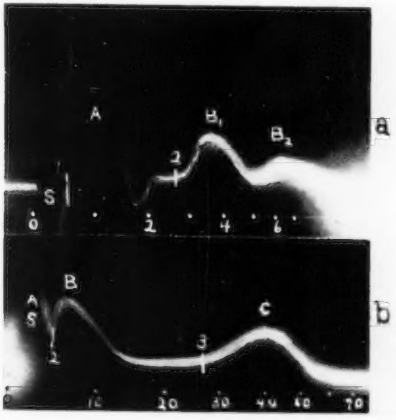

Fig. 4. Records, retouched, from bullfrog skin nerve, 11/20/28; slightly diphasic. Distance of conduction 0.9 cm. Temp. 22°C. 4-panel amplification. × 3/4.

a, 5,000ω, stimulus maximum for *B*.

b, 45,000ω, stimulus maximum for *C*. A third record of the series taken at less amplification shows that the height of *A* on the scale of these records is 152 mm. The conduction rates of *A*, *B* and *C* are 20, 3.4 and 0.35 m.p.s.

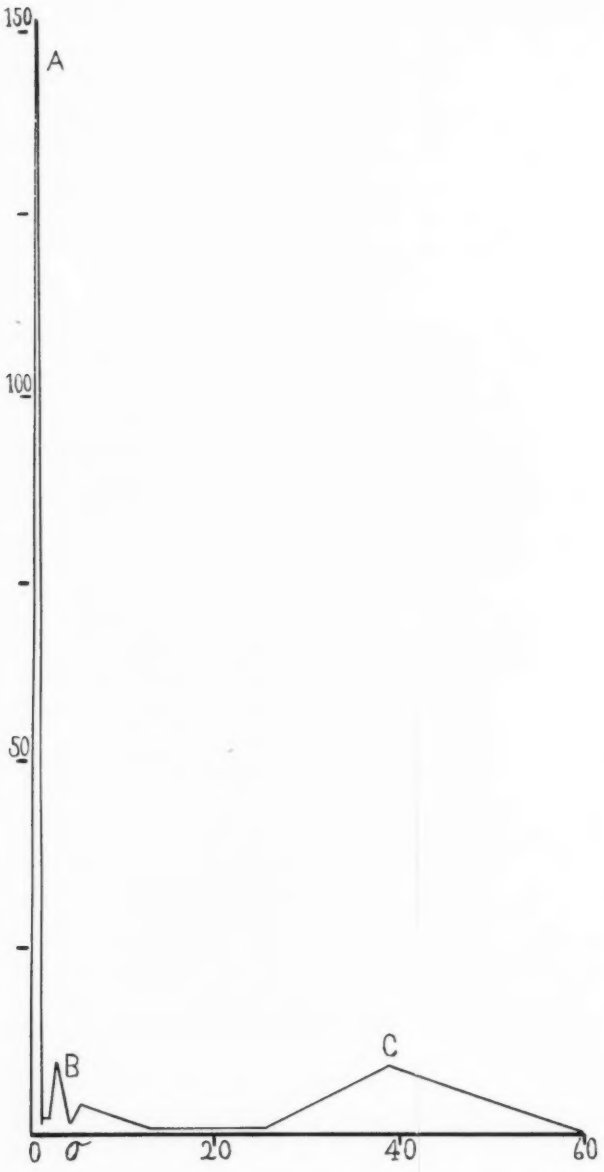

Fig. 5. The action potentials of figure 4 (bullfrog skin nerve) transferred to linear coördinates as in figure 3.

very high narrow spike at the left edge of the chart, the *C* elevation a low broad lift far to the right, while the *B* elevation consists of a peak of intermediate height and width a little to the right of the *alpha-beta-gamma* (*A*) spike.

Three elevations, comparable with these sciatic waves as regards rates of conduction and relative irritabilities, are exhibited by all cold-blooded somatic nerves we have examined. These include the dorsal skin nerve in the bullfrog (figs. 4 and 5), the sciatic and the branch of the sciatic to the gastrocnemius muscle in the green frog, and the sciatic of the turtle (Heinbecker[4]). Waves, presumably comparable, have been observed also in the tibial, peroneal and saphenous nerves in the dog (see figs. 6 and 7) and cat. In warm-blooded muscle nerves the *B* elevation may be small, as in muscle branches of the dog's femoral (see fig. 6), or practically absent, as in the phrenic nerve of the dog. The significant data from many of these observations are collected in table 1. The *B* and *C* waves often are double, particularly the *C* elevation, and then the position of the cleft is not constant in the different preparations. The *C* elevation may have the form of a plateau which may be level or slightly undulating, and often is higher at the late end than the front. The very strong shocks needed to stimulate the *C* fibers rapidly cause deterioration of the nerve under the electrodes, and it is, therefore, difficult to obtain exactly comparable successive records of *C*. The height of the *B* and *C* waves in a given nerve depends in part upon the distance of conduction; like the *A* elevation (Erlanger, Gasser and Bishop, 1924), they become lower and longer as they move away from the site of their origin.

INDIVIDUALITY OF A, B AND C ELEVATIONS. The elevations we have designated *A*, *B* and *C*, like the *alpha*, *beta* and *gamma* waves of *A*, unquestionably are produced by a grouping of conduction rates in as many different sets of fibers composing the nerve. The only other possibility is that they are successive waves started in the same fibers by the strong shocks needed to elicit the slower waves. Repeated stimulation of the same fibers by a single shock, sometimes at the cathode, sometimes at the anode (Heinbecker, 1928), occasionally does occur, but repetitive waves usually are readily recognizable as such in the oscillograph picture. That the *B* and *C* elevations are not repeating action potentials can be demonstrated by the crucial test that supplied the proof of the individuality of the fibers producing the *alpha*, *beta* and *gamma* waves (Erlanger, Gasser and Bishop, 1924). When an action potential, started from one end of a nerve by a

[4] The *C* wave in mixed nerve was found independently by Heinbecker while working on the cervical sympathetic nerve of the turtle under the direction of one of us. Elevations corresponding, presumably, to *B* and *C* of somatic nerves have been described by Heinbecker and Bishop (1929) as occurring in the cervical sympathetic and vagus nerves of both cold- and warm-blooded animals.

TABLE 1

PREPARATION	CONDUCTION DISTANCE	CONDUCTION RATES IN M.P.S. OF FIBERS OF						RELATIVE AMPLITUDES			TIME TO MAXIMUM			DURATION			REMARKS
		A		B		C											
		First	Last	First	Last	First	Last	A	B	C	A	B	C	A	B	C	
	cm.										σ	σ	σ	σ	σ	σ	
1. Green frog peroneal 11/13/28	2 4	—	—	2 6	1 04	0 45	0 13	—	5 7	1	—	4 46	15 5	—	13 81	132 3	Notch of C 0.23 m.p.s. Maxima, B 11 5, C 3 cm. Duration to notch 44 0
2. Green frog peroneal 1/5/29	3 12	36 1	18 4	3 08	1 26	0 47	0 14	65 4	1 4	1	0 78	4 94	15 9	2 63	15 16	147 0	Maxima, B 7 4, C 0 8 cm.
3. Green frog peroneal 3/21/29	2 8	30 6	—	3 33	1 71	0 57	0 29	142 0	5 2	1	—	5 7	25 65	—	12 437	47 65	
4. Bullfrog sciatic 11/13/28b	4 0	—	—	4 2	1 4	0 71 0 56	— 0 30	—	6 0	1	—	7 45	16 5	—	19 5	79 5	Notch of C 0 46 m.p.s.
5. Bullfrog sciatic 11/13/28c	3 3	—	—	—	—	0 65	0 24	—	6 4	1	—	—	38 5	—	—	90 0	
6. Bullfrog sciatic 11/20/28b	5 8	48 7	13 8	5 3	1 8	0 66	0 37	163 0	4 7	1	0 75	6 5	26 4	3 6	22 35	68 5	
7. Bullfrog sciatic 11/20/28c	6 4	—	—	—	—	—	—	—	3 8	1	0 49	9 04	42 1	α1 35 β1 66	17 6	77 6	Constant current; conduction rates not determinable
8. Bullfrog sciatic 1/29/29 a	7 4	55 3	11 9	4 48	1 58	0 54	0 27	54 6	1 9	1	0 69	8 89	35 5	5 67	31 12	146 0	Maxima, B 9 4, C 5 2 cm.
8. Bullfrog sciatic 1/29/29 b	7 4	48 0	11 7	4 06	1 45	0 67	0 29	64 6	2 3	1	—	7 34	53 5	5 67	33 55	146 7	Maxima, B 8 8, C 5 2 cm.
9. Bullfrog sciatic 1/31/29	9 1	42 0	13 0	4 40	1 73	0 56	0 29	43 4	2 5	1	0 87	9 17	39 5	5 31	32 65	150 2	Thresholds, A 25 5, B 11 8, C 7 6 cm. Maxima, B 7 5, C 3 9 cm.
10. Bullfrog skin nerve 11/20/28	0 9	20 0	5 5 6 1	3 05 3 4	—	0 35	0 15	17 4	1 06	1	—	—	—	0 97	6 19	34 12	B notch 1 7 m.p.s. Duration to notch 2 25
11. Bullfrog skin nerve 1/5/29	1 53	23 3	6 53	3 42	1 19	0 39	0 23	48 5	3 0	1	0 74	6 50	9 68	2 66	19 24	27 0	Maxima, B 20 2, B 7 8, C 0 5 cm.
12. Dog femoral 11/2/28 Muscle	9 0	85 7	—	—	—	—	—	26 0	1	—	—	—	—	—	—	—	
12. Dog femoral 11/2/28 Saphenous		69 2	39 1	16 2	10 3	—	—	18 0	1	—	—	2 1	—	1 6	3 8	—	

13. Dog femoral 11/10/28	Muscle	6 7	82 7	—	—	—	—	—	—			—	—	—	—	—	—	No B in muscle-nerve
	Saphenous		70 8	55 2	13 1	10 2	—	—	22 0	1	—	—	1 09	—	—	2 3	—	
14. Dog femoral 11/14/28	Muscle	4 4	77 0	—	—	—	—	—	—	—	—	—	—	—	0 92	—	—	Trace of B in muscle-nerve
	Saphenous		58 7	36 4	11 2	8 33	—	—	48 0	1	—	—	0 92	—	1 06	1 96	—	
15. Dog femoral 11/14/28, Saphenous		4 9	77 0	49 0	13 9	10 1	—	—	—	—	—	—	—	—	—	—	—	
16. Dog femoral 11/24/28, Saphenous	a	12 0	80 0	35 2	16 8	13 2	1 04	0 8	79 5	14 0	1	—	1 09	14 4	2 51	2 53	34 4	In situ
	b	9 0	61 0	29 3	14 2	11 8	0 91	0 7	8 5	1	—	—	0 67	11 9	2 2	1 9	30 3	
17. Dog femoral 11/26/28, Saphenous	a		63 8	34 7	13 1	—	1 28	0 57?	61 7	9 5	1	—	1 0	15 2	1 38	1 43?	58 4	Duration to notch of C 23 7
	b	5 9	62 8	34 7	13 9	10 98			—	—	—	—	—	—	1 36	1 75	—	Maxima, B 11 1, C 5 0 cm.
18. Dog femoral 1/30/29	Muscle	6 95	89 6	55 2	Trace		Small		—		—	0 39	—		1 19	—	—	Maxima, B 14 8, C 7 1 cm.
	Saphenous		73 2	47 6	20 0	15 3	1 54	0 67	32 8	4 3	1	0 39	0 44+	17 7	1 16	1 66	59 2	
19. Cat 5/1/29 Saphenous		1 55	67 0	?	14 6	9 22	1 19	0 64	6 0	1	>1	—	—	—	—	—	—	Thresholds, A 31 9, B 18 4, C 7 5 cm.
20. Cat 5/1/29 Saphenous		5 5	88 5	37 7	20 6	16 8	1 46	1 03	5 4	1	>1	—	—	—	—	—	—	Thresholds, A 33 9+, B 21 3, C 7 7 cm.
21. Cat peroneal		2 9	93 6	?	32 3?	16 4	1 59	0 82	20 0	1	>1	—	—	—	—	—	—	Thresholds, A 33 9+, B 21 3, C 8 1 cm.
22. Dog phrenic 4/16/27		3 75	55 2	—	11 0?	—	1 13	—	18 0?	0 07	1	—	—	—	—	—	—	Thresholds, A 33 5, B 16 0, C 8 9 cm. In two other nerves B was imperceptible

stimulus just below the threshold, of the *B* elevation, for instance, is made to meet an action potential consisting of both the *A* and the *B* elevations started from the other end of the nerve by a stimulus of appropriate strength, the *A* elevation of the latter is blocked while the *B* elevation passes on without modification. Or, by stimulating a nerve[5] first with a shock that is maximal for *C* and again, at the same place, after the *A* fibers have recovered from their refractory state, with a shock maximal for *A* one can start one or more *A* waves that will overtake the *C* wave and beat it to the lead. The configurations of the waves, *A* and *C*, are unaffected by this interesting process.

SURVEY OF THE RELATION OF AXON CONDUCTION RATE TO THE CONFIGURATION OF THE ACTION POTENTIAL. The positions taken by the three elevations, *A*, *B* and *C*, in the records, and their durations, are, of course, determined mainly by the propagation rates of the contributing fibers and by the distance of conduction. The rate of movement of the head of a wave is determined by the rate of conduction in the fastest fiber contributing to the potential of that wave. Our method of determining it, namely, as the time intervening between the shock and the beginning of the elevation, is subject to two errors; it fails to take cognizance either of the utilization period of the shock, which, however, is so short as to be almost negligible even in the case of the fast moving *A* elevation (Erlanger, Gasser and Bishop, 1924), or, when superthreshold shocks are used, of the spread of the stimulus. With threshold shocks the spread will be negligible, but with strong shocks it may, in the case of the most irritable fibers, extend out a distance of 1 cm. or more (Erlanger, Gasser and Bishop, 1924). When, as in many of the present experiments, the rates have been derived from records obtained by stimulation strong enough to elicit the *C* elevation it is obvious that the deduced conduction rates of *B*, and especially of *A*, may be somewhat too fast, relatively. Only, however, when the conducting distance is short will the error from this source become considerable.

The conduction time of the slowest fiber contributing to a wave can be arrived at approximately by deducting from the conduction time of the end of the wave the duration of the axon action potential. It has been assumed, in doing this, that the potentials in the axons contributing to the *B* and *C* elevations have the same duration as in those contributing to the *alpha*, *beta* and *gamma* waves, namely, 0.9 and 0.6 σ in cold- and warm-blooded nerve, respectively (Gasser and Erlanger, 1927). Should it eventuate, as Heinbecker's preliminary observations seem to indicate (1929), that the action potentials of some or all of the slower fibers are longer, the corrections necessitated would not materially affect the deductions we are making; for the slower elevations, excepting warm-blooded *B*,

[5] For this test a bullfrog's motor root was used.

TABLE 2

Table of limiting rates in m.p.s. (measured), and fiber sizes in μ (estimated on basis of proportionality of fiber diameter to conduction rate)

			A		B		C	
			First	Last	First	Last	First	Last
Sciatic, bullfrog	Rate		42	13 to 10	5.4 to 4.0 ave. 4.53	1.8 to 1.26	0.71 to 0.46 ave. 0.59	0.37 to 0.23
	Size		20	6 to 5	2.6 to 1.9	0.9 to 0.6	0.34 to 0.22	0.18 to 0.11
Skin nerve, bullfrog	Rate		30		3.42 to 3.05	1.19	0.39 to 0.35	0.23 to 0.15
	Size		14		1.59 to 1.42	0.55	0.18 to 0.14	0.11 to 0.07
Peroneal, green frog	Rate		30		3.29 to 2.60	1.26 to 0.96	0.47 to 0.28	0.14 to 0.10
	Size		14		1.5 to 1.2	0.6 to 0.4	0.22 to 0.13	0.07 to 0.05
Femoral preparation, dog	Rate	M*	82.8					
		S*	65.6	55.2 to 29.3 ave. (10) 38.91	16.8 to 11.2 ave. (10) 13.83	13.2 to 8.3 ave. (8) 10.5	1.28 to 0.82 ave. (8) 0.97	0.80 to 0.33 ave. (7) 0.58
	Size	M	17					
		S	13.5	ave.† 7.98	3.45 to 2.30 ave. 2.84	2.7 to 1.7 2.2	0.26 to 0.17 0.20	0.16 to 0.07 0.12
Femoral preparation (P), dog, 1/30/29	Rate	M	89.6					
		S	73.2	47.6	20.0	15.3	1.54	0.67
	Size	M	17					
		S	13.9	9.04	3.80	2.90	0.29	0.13

* M = Muscle branch, S = Saphenous.

† End of first large deflection.

are relatively long in duration, and their ends cannot be located with any great precision. The rates in the fastest and slowest fibers of *A*, *B* and *C* thus determined in certain of the nerves are included in table 1 and summarized in table 2. The limiting conduction rates of the *A* elevation have not been determined as a routine in the present investigation. For the purposes of this paper the numerous determinations we have made in previous studies are available.

In the *bullfrog sciatic* the normal rate of conduction, at room temperature (usually 23–25°C.), in the axons forming the head of the first, or *A*, elevation may be taken as 42 m.p.s., though rates as high as 48 m.p.s. are occasionally observed, and of the end of *gamma*, a rather indefinite point, as 13 to 10 m.p.s. For the *B* elevation the corresponding values are, start, 5.4 to 4.0, end, 1.8 to 1.26 m.p.s.; and for the *C* elevation start, 0.71 to 0.46, end, 0.37 to 0.23 m.p.s. On the basis of proportionality of fiber diameter to conduction rate (Gasser and Erlanger, 1927), if 20μ be taken as the diameter of the largest fiber in the bullfrog's sciatic, the diameters of the fibers conducting at the other rates would be (see table 2), in the order above used, *A*, end, 6μ; *B*, start, 2.6 to 1.9, and end, 0.9 to 0.6 μ; *C*, start, 0.34 to 0.22, and end, 0.18 to 0.11 μ. Since myelinated fibers practically never are less than 1.5μ in diameter and rarely less than 2μ, it follows that the fibers producing the *C* elevation certainly, and the *B* elevation probably, fail to obey the fiber-size rule.

Similar analysis of the action potentials of the other nerves examined leads to the same conclusion. Thus, a *skin nerve of the bullfrog* ordinarily conducts at rates ranging between 26 and 35 m.p.s. and its largest fiber measures something like 14μ (Erlanger, 1927). The diameters of the fibers determining the limiting rates of its slower waves, taking 30 m.p.s. as the rate in a 14μ fiber, become, on the basis of the present data, for the *B* elevation, start, 1.59 to 1.42μ, and end, 0.55μ; for the *C* elevation, start 0.18 to 0.14, and end, 0.11 to 0.07μ.

In the case of the *B* elevation of the *sciatic nerve of the green frog* the limiting fiber sizes taking the conduction rate in the largest fiber (14μ) as 30 m.p.s. (Gasser and Erlanger, 1927), on the basis of our data would be, start, 1.5 to 1.2μ, end, 0.6 to 0.4μ. For the *C* elevation the fiber diameters would be, start, 0.22 to 0.13, and end, 0.07 to 0.05μ.

Of warm-blooded nerves the *saphenous and muscle branches of the femoral nerve of the dog* have been singled out for a more complete study of the relation of fiber-size to conduction rate. This has been done because, by using as the preparation the trunk with attached saphenous nerve and some one muscle branch, and by recording from each of these two branches separately the action potential started by stimulation of the trunk, exactly comparable records can be obtained from a purely cutaneous nerve and a purely muscle nerve (Erlanger, 1927). The femoral nerve itself is so short

and thick that a good mixed-nerve action potential at a sufficient distance from the site of stimulation cannot be obtained from it for comparison with the separate action potentials of its branches. There is every reason for

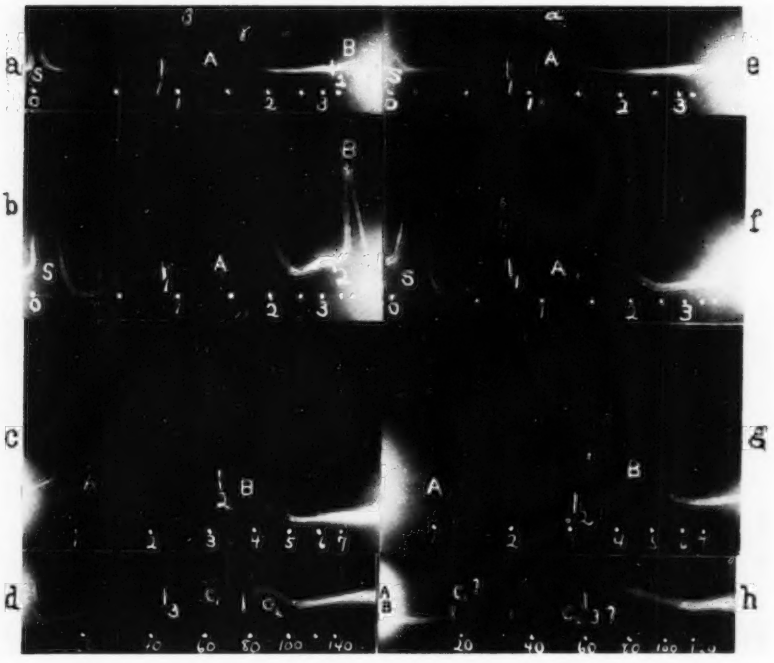

Fig. 6. Records from a femoral, saphenous and muscle-branch preparation, 1/30/29 (retouched). Distance of conduction 6.95 cm. Temp. 37.5° C. Stimulus to femoral nerve in all cases. × 3/4 approx.

a. Saphenous. 3 panels, 2,000ω. Stimulus maximum for *B*, coil at 13.4 cm.

b. Saphenous. 4 panels, otherwise same as *a*.

c. Saphenous. 4 panels, 4,000ω. Stimulus maximum for *B*, coil at 14.8 cm.

d. Saphenous. 4 panels, 92,000ω. Stimulus maximum for *C*, coil at 7.1 cm.

e. Muscle branch, otherwise same as *a*.

f. Muscle branch. 4 panels, otherwise same as *a*.

g. Muscle branch, otherwise same as *c*.

h. Muscle branch, otherwise same as *d*. Conduction rates: *A* of muscle branch 89.6, of saphenous 73.2; *B* of muscle branch (?), of saphenous 20.0; *C* of muscle branch 4.44 (?), of saphenous 1.5 m.p.s.

believing, however, that the femoral action potential is formed by a combination of action potentials similar to those of these two of its branches. Records from longer mixed nerve, the peroneal of the cat, for example, show that this is the case (see table 1).

The data derived from the femoral nerve of the dog are summarized in table 2. The values there averaged include all excepting those obtained from the nerve of the experiment dated 1/30/29. The latter nerve (for the sake of convenience, it will be referred to as *P*) is treated separately because the conduction rates derived from it are decidedly the fastest of the series. Whether, on this account, they are to be regarded as the nearest to normal is a question that must be left open. All that can be said is that in every other respect the records from this preparation are typical; those of them that are required for a complete story, given in the legend, are included in figure 6. Rates in the saphenous of the cat often are as high as in this case.

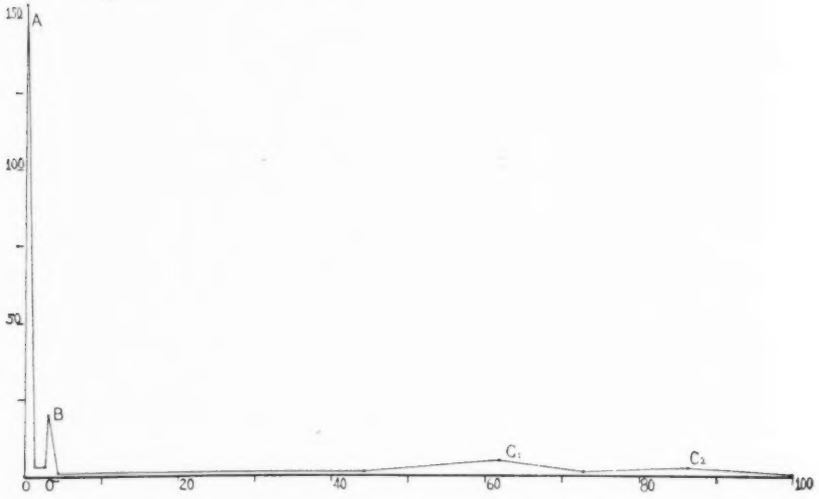

Fig. 7. The saphenous action potentials of figure 6 transferred to linear coördinates as in figure 3.

In these femoral preparations the beginning of the muscle branch action potential has traveled at rates ranging between 85.7 and 77.0 m.p.s., the average being 82.8 (in *P* the rate was 89.6). In the case of the saphenous branch the rates have ranged between 80.0 and 58.4 m.p.s., the average being 65.6 (in *P*, 73.2). The slowest axon action potential of the *A* elevation travels at rates that have ranged between 55.2 and 29.3 m.p.s., with an average of 38.9 (in *P*, 47.6). Taking 17μ as the diameter of the largest fiber of the femoral nerve (Erlanger, 1927), the smallest fiber of the *A* elevation is found by calculation to range between 11.3 and 6.2μ, with the average at 7.98μ;[6] the smallest *A* fiber in *P* would measure 9.04μ. The

[6] The slowest fiber contributing to the main elevation of the saphenous action potential reconstructed by Erlanger (1927) has an estimated diameter of 7.25μ.

range of axon conduction rates of the fastest fiber of the *B* elevation is 16.8 to 11.2 m.p.s. (average of 10 determinations, 13.8, and in *P*, 20 m.p.s.), of the slowest fiber, 13.2 to 8.3 m.p.s. (average of 8, 10.5, and in *P*, 15.3); the range of axon conduction rates of the *C* elevation is, fastest, 1.28 to 0.82 (average of 8, 0.98, and in *P*, 1.54), and of the slowest, 0.80 to 0.33 (average of 8, 0.58, and in *P*, 0.67). The fiber sizes calculated from these rates are for *B*, largest, 3.45 to 2.30μ (average 2.83, and in *P*, 3.80), smallest, 2.71 to 1.70 (average, 2.16, and in *P*, 2.90); and for the *C* elevation, largest, 0.26 to 0.17 (average, 0.20, and of *P*, 0.29), smallest, 0.16 to 0.078 (?) (average, 0.12, and in *P*, 0.13). Here again the result of calculation is such as to force the conclusion that proportionality of conduction rate to fiber diameter cannot possibly hold in the case of the fibers forming the *C* elevation and probably does not in the case of the fibers forming the *B* elevation.

RELATION OF THE B AND C POTENTIAL WAVES TO THE MORPHOLOGY OF THE NERVE. The problem is to find on histological examination the fibers serving as the source of the potential. The method of approach is to take the fiber distribution, found on analysis, and attempt to reproduce the potentials recorded, using the assumptions which were successful in the reconstruction of the *A* wave, namely, that the axon potential has the same form and duration in all fibers, that the velocity of conduction is in linear relationship to the fiber diameter, and that the fibers affect the recording instrument as their cross-sectional areas (Gasser and Erlanger, 1927). This necessitates a complete analysis of the small fibers in the nerve. The only nerve in which this had previously been done was the skin nerve of the frog, although the small fibers in the saphenous of the dog had been counted (Erlanger, 1927). In most nerves the measurements had stopped in the region of 3–5μ, and it was, therefore, necessary to complete them.

Since our most complete data have been derived from the saphenous nerve of the dog this will be considered first. The saphenous nerve contains so many fibers that to map it completely is a stupendous task and since "the large myelinated, the small myelinated and the unmyelinated fibers are not uniformly distributed" (Langley, 1922) anything short of a complete analysis of a nerve may fail to produce a reliable picture of the whole.

In an effort to meet this difficulty we have completely analyzed a small branch of the saphenous nerve (1/30/29) from which the records of figure 6, *a*, *b*, *c* and *d*, were obtained. This branch arose about 1 cm. beyond the point from which the lead was taken; it was placed in osmic acid at the time the nerve was mounted for observation. It contained 341 myelinated fibers and a considerable amount of unmyelinated material (possibly half the area of all the fibers), all beautifully prepared. The measurements, made on a photograph enlarging the fibers over 2,000 times, were controlled by

measurements with an eyepiece micrometer and an oil immersion lens. In comparing this analysis with the action potential it is assumed that the branch is a fair sample of the trunk and would yield a wholly comparable action potential.

The action potential reconstructed from the values obtained from the measurement of the fibers of this nerve as of 69.5 mm. of conduction, that is, the conducting distance at which the records of figure 6 were obtained, is shown in figure 8. Onto this figure have been transferred as triangles the waves of figure 6 in correct time and height relations.

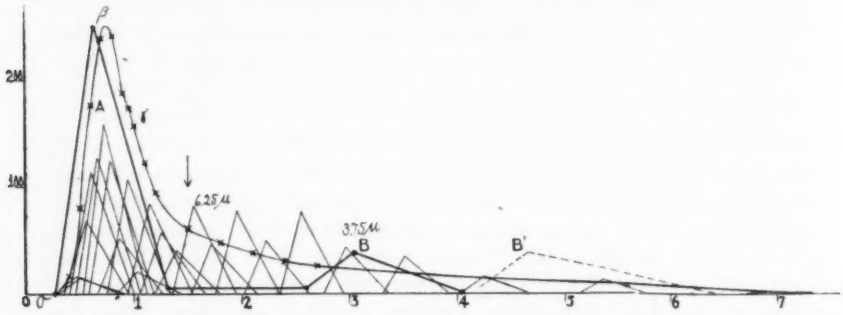

Fig. 8. Reconstruction of the dog's saphenous action potential (crosses) as of 6.95 mm. conduction, based on the histological analysis of a branch of the nerve from which the saphenous action potentials of figure 6 were obtained. Zero lag is that of the fastest axon action potential of the femoral, saphenous and muscle-branch preparation. The recorded saphenous action potential is superimposed in the same scale by joining with straight lines the dots marking its significant points, the recorded β being given the same height as the reconstructed β; the heights of other waves are drawn relative to this. The arrow indicates the position of the end of A in the average saphenous action potential. The average position of B is indicated by dotted elevation, B'.

As has always been the case the main, or A, wave of the reconstruction and of the record are essentially alike, the times to maximum being respectively 0.48 ($\pm$0.15) and 0.39 σ, and what may be taken as the duration, 1.2 ($\pm$0.1) and 1.11 σ, respectively. But beyond this the agreement, as heretofore, ceases. The reconstruction fails to bring out any other elevation than A, showing merely a very definite flattening of the decline beginning with what we take to be the end of the main wave, coincident with the position of the triangle representing the 6.25μ fiber. The recorded B elevation of nerve, P, travels at about the theoretical rate of a 3.75μ fiber, and the B elevation (dotted), traveling at the mean saphenous B rate moves at the rate of a 2.75μ fiber.

This reconstruction is quite in accord with the previous one in which

the small fibers were only counted (Erlanger, 1927). To produce an elevation simulating *B* the nerve would have to exhibit an accumulation of fibers between 3.45 and 1.7μ with a mean at 2.8μ. Such is not the case. All sizes of fibers are represented while there is a gap in the recorded potential.

The *skin nerves* of the *bullfrog* are so small—they may contain fewer than 150 fibers measuring more than 3μ in diameter—that to map completely all of the fibers is not a particularly arduous task. The main

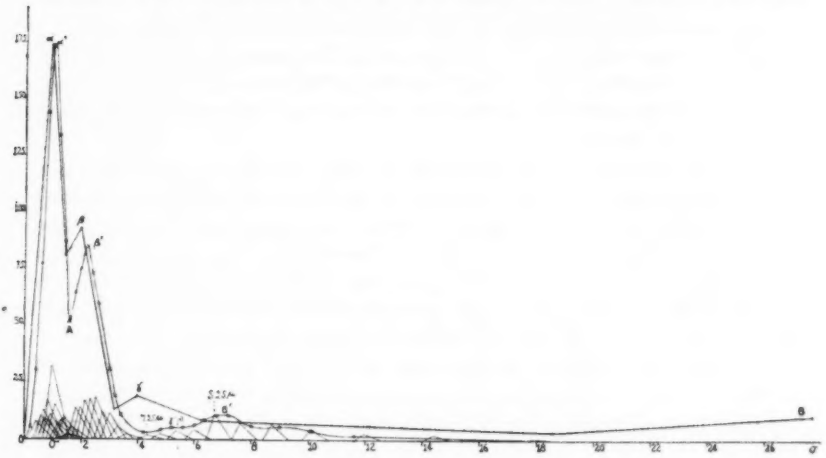

Fig. 9. Reconstruction of the bullfrog's sciatic action potential (the one shown in figures 10 and 11, Gasser and Erlanger, 1927) as of 9.1 cm. conduction, zero being the position taken by the start of the fastest axon action potential (traveling 42 m.p.s. in a 20.75μ fiber). Superimposed on this is the action potential of figure 1 as straight lines joining the significant time points. If α, β, γ and B of the recorded action potential are produced by the fibers of α', β', γ' and B' of the reconstruction it would follow that conduction in the γ fibers is slightly out of accord with the fiber-size rule, and in the B fibers entirely out of accord. The area of A is about the same as that of A', but the area of B is more than twice that of B'.

difficulty here, inexplicably, has been in getting a preparation with uncrenated fibers. The best preparation we have succeeded in obtaining, after several attempts, is the one used as the basis of the chart and reconstruction already published (Erlanger, 1927, figs. 7 and 8). On account of the increased interest the smaller fibers have assumed, the smaller myelinated fibers of this section have been remeasured as accurately as the crenation would permit, this time not only from the enlargement but also with an oil immersion lens and eyepiece micrometer. The result has been not to alter materially the distribution of the myelinated fibers in the chart. If

the last three triangles in the chart be omitted, there remain only those which represent myelinated fibers. Accordingly a reconstruction was made of the potential, which would be produced by myelinated fibers alone. In this reconstruction a wave developed resembling *B*, in figure 4, but it was in such a position as to indicate a velocity of 6.5 m.p.s. instead of the observed velocity of 3.4 m.p.s. This discrepancy is too great to be accounted for by an error in either measurement involved.

Analysis of the data derived from the *sciatic and peroneal nerves of the bullfrog* and of the *green frog* leads to much the same result. The preparation on which figure 10 (Gasser and Erlanger, 1927) was based was very suitable for further analysis. Consequently the measurements were completed so as to include all the small myelinated fibers in the area examined; and the measurements, as in the case of the other nerves, were subjected to microscopic control.

The result of this analysis is included in figure 9 in such a way as to make possible comparison between the reconstructed action potentials (crosses) and the action potential of figure 1 (dots). As has always been the case the correspondence as regards *alpha* and *beta* is very close indeed; the correspondence with *gamma* is rather poor, but there is an indication of such a wave in the reconstruction, and the resemblance would probably be still better if the *alpha* and *beta* potentials had been drawn with the retention of potential which follows the fast part of the axon potential represented by the triangles. The *gamma* potential would then be found on a higher base line than appears in the reconstruction. A comparison of the *gamma* wave and the third pile of fibers in the distribution curve brings out one point of considerable importance. The wave can at best utilize only a small part of the third pile. The area of the fibers is too great and their potentials appear too late in the theoretical reconstruction.

Now, can the fibers not needed for the *gamma* wave be made to account for the *B* wave? Certainly not without departing from the assumptions made at the beginning of this section. The recorded wave, *B*, begins 12 σ later than the only elevation, B', in the reconstruction that could be assigned to *B*. In fact, the recorded *B* actually begins later than the arithmetically derived action potential in the slowest myelinated fiber of the nerve.

The foregoing observations show clearly that the velocity of conduction in the small fibers is slower than the one calculated on a size basis from the velocity in the fastest fibers. It remains to be ascertained whether signs of other differences may be found.

It is reasonable to suppose that the potential in the late waves must be derived from the third pile of fibers and from the unmyelinated fibers. If these fibers have the same axon action potential form as the large fibers it is easy to calculate the potential area they could yield; and this area

can then be compared with the one experimentally observed. Unfortunately a technical difficulty lies in the way of accurate determination of the areas of the late waves in comparison with the *A* wave. The shock artefact, which is large on account of the strong stimuli, the retention of potential from the *A* wave, and the greater tendency toward diphasicity introduce an uncertainty as to the position of the base line, which cannot be accurately corrected; and this uncertainty introduces considerable error into the measurements of the area of waves of low potential and long duration. Therefore, in transferring the waves to a linear coördinate system prior to the measurement of the areas we have not taken the trouble of carrying over the exact form but have merely employed triangles drawn in their proper time and amplitude relations. The values we have obtained are included in table 3. It is not surprising under the circumstances that

TABLE 3

Table of areas (arbitrary units) of the A, B and C elevations

	A	B	C	$\frac{B}{A}$	$\frac{C}{A}$
				per cent	*per cent*
1. Bullfrog sciatic of figures 1, 3 and 9	1,266	693	2,700	54·8–40·0	213·0
2. Bullfrog sciatic 11-20-28; approximate	1,178	350++	315	29·7	26·7
3. Bullfrog skin nerve of figures 4 and 5	454	105	752	23·1	12·3
4. Dog saphenous (11-24-28)	1,240	227	215	18·3	17·3
5. Dog saphenous of figures 6, 7 and 8	710	160	789+++	22·5	111·0

they vary irregularly among themselves; they can only be considered as approximations.

The area of the *B* elevation in figures 6 and 8 is 22.5 per cent of *A*. The area of all the fibers in the nerve measuring 6.25μ and less is 29 per cent of those larger than this. There is thus sufficient area to account for *B;* but there is then very little left over for *C*, which may also occur, at least in part, in myelinated fibers, as will be indicated in a later part of the paper. In the frog skin nerve the area of the entire pile of small myelinated fibers, which includes the fibers measuring 4.25μ and less, is 14.9 per cent of the area of the fibers measuring 4.75μ and more. The *B* wave in figures 4 and 5 has an area 23 per cent of the *A* wave, therefore the small myelinated fibers would not supply the *B* wave alone. The same is true in the sciatic. The *B* wave in figures 1 and 9, after deducting the largest possible error due to the uncertainty of the position of the base line, is 40 per cent of the area of *A*, while the fibers below 7.25μ have an area only 18 per cent of those above.

The result of the foregoing attempt to reconstruct the action potential of the *B* and *C* waves from the histological picture, using the constants of the *A* wave, is to show that it is impossible. Therefore the constants, which have been determined for the axon potentials of the *A* wave fibers, cannot apply to the potentials developing in the majority of the fibers in the last pile.

SOURCES OF THE A, B AND C FIBERS. Somatic nerves acquire fibers by way of the dorsal and ventral roots and the gray rami communicantes, and the next step in the investigation consisted, therefore, in ascertaining which of the elevations of the action potential occur in these structures and which of them pass on into the somatic nerves.

SPINAL ROOTS: *Bullfrog.* Previous work on the bullfrog (Erlanger, Bishop and Gasser, 1926) has shown that the dorsal roots contribute to peripheral nerves an *A* elevation consisting of *alpha*, *beta* and *gamma* waves and the ventral roots an *A* elevation consisting of *alpha*. Stronger stimulation and higher amplification now reveal in the action potentials of the roots elevations traveling much more slowly than *A*.

Excised dorsal roots. Strong stimulation of the roots examined (6th to 9th inclusive)[7] elicits an elevation in the action potential (see fig. 10, *a*, *b*, and *c*) that travels at rates usually ranging between 0.4 and 0.5 m.p.s., rarely as slowly as 0.3 m.p.s. or as rapidly as 0.6 m.p.s. (at room temperature, usually 23 to 24°C). To obtain these elevations in maximum amplitude the secondary coil must be set at 4 to 6 cm.; and the threshold usually is in the vicinity of 7 to 8 cm. Obviously as regards conduction rate and irritability these waves belong in the category of the *C* elevation of mixed nerve. The *C* action potentials appear in many configurations even at the short distances of conduction to which the observations are limited, usually 6 to 12 mm. (see fig. 10, *b* and *c*). Usually the elevations are rather long drawn out, and often are compounded of 2 or 3 waves; more usually they are plateau shaped. The action potentials of the roots of a pair may be strikingly alike in appearance. Calculation in a few cases in which *C* was well developed indicates that it may have as much as 15 to 30 per cent the area of *A*. Owing to the rapid deterioration of the elevations that results from the strong stimuli needed to elicit them, and to the very much greater diphasicity of *C*, these results necessarily are extremely inaccurate; but, if anything, *C* suffers more than *A* in the comparison because the record of *A* is made before the nerve is strongly stimulated for *C*.

There is no definite and constant elevation in the dorsal root cor-

[7] We are numbering the roots as in our previous publication, though we have since learned that what is regarded in other animals as the first spinal root is atrophic in the frog. From the developmental standpoint, therefore, the numbers by which we designated frog roots are too small by one.

responding in rate of conduction and irritability with the *B* elevation of mixed nerve.

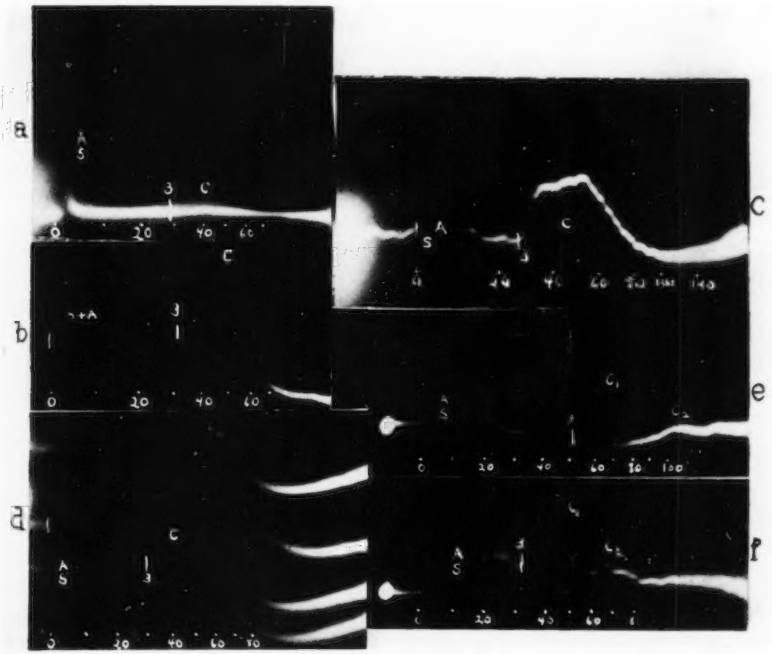

Fig. 10. Dorsal root action potentials of the bullfrog. × 2/3.

a, 7th root (4/20/29). 3 panels but shunted slightly to bring *A* within range of film. 33,000ω. Conduction distance 0.65 cm. Stimulus maximum for *C*. *C* rate 0.4 m.p.s.

b, Same as *a*, but with unshunted 4-panel amplification, i.e., more than 18 times *a*. Single transit.

c, 9th root (4/20/29). 4 panels, 72,500ω. Conducting distance 1.2 cm. Stimulus maximum for *C*. Rate of *C* 0.47 m.p.s. Single transit.

d, 9th root (4/10/29) stimulated, lead from trunk central to rami. Three single transits of spot on one film. 4 panels, 95,000ω. Conduction distance 1.31 cm. Stimulus maximum for *C*. *C* rate 0.4 m.p.s. The base line is badly deformed by the shock artefact. The time marks apply to the middle deflection.

e and *f*, 7th trunk (7/11/29) stimulated, lead from dorsal root. 4 panels, 233,700ω. 1/2 mf. 28.5°C. Single transits by the newest technique.

In *e* the stimulus was applied distal to attachment of rami. Conduction distance 4.0 ± cm. Stimulus maximum for *C*. Rates, $C_1 = 0.74$, $C_2 = 0.45$ m.p.s.

In *f* the stimulus was applied central to attachment of rami. Conduction distance 2.0 ± cm. Stimulus maximum for *C*. Rates, C_1 0.62, C_2 0.39 m.p.s. Ratio of areas of C_1 in *f* and *g* is 1:1.5.

The dorsal root C wave in the trunk. Records have been made of the action potentials traveling in either direction through preparations consisting of the dorsal root and the nerve *trunk* formed by its union with the ventral root.[8] These preparations either have been removed from the body clear of all other tissues as in previous studies (Erlanger, Bishop and Gasser, 1926) or, more usually, they have been removed along with the tissues of the back through which the roots pass on their way from the vertebral canal to the subperitoneum. Excepting the small dorsal branch and the rami communicantes, the preparations are branchless. Observations have been confined to the 7th, 8th and 9th roots.

The records (see fig. 10, *d*, *e*, *f*) show that the dorsal root *C* elevation travels the whole length of the preparation in either direction and without any material changes in the form other than those attributable to the changing phase relations of the action potentials traveling at different rates in the contributing axons. The areas of the action potentials recorded in the root and started by stimulation of the trunk, first, peripherally of, and then, centrally to the junction point with the rami communicantes cannot be measured with sufficient accuracy (see fig. 10, *e* and *f*) to be of any material assistance in reaching a decision as to whether or not any of the *C* fibers of the dorsal root leave via the rami. It is only possible to say, on the basis of such measurements, that if any fibers at all leave, the maximum cannot exceed a third. Direct evidence that few if any of the dorsal root fibers leave the trunk by way of the white ramus has been obtained by stimulating the 7th dorsal root while leading from the 7th rami. In such experiments no perceptible deflection has been obtained in the case of preparations known to be sensitive, the posterior roots having subsequently yielded good *C* elevations in direct leads.

The *A* action potential, it has been shown (Erlanger, Bishop and Gasser, 1926), suffers a delay of about 0.14 σ in passing through the posterior root ganglion. The *C* fibers conduct so slowly that a lag of this magnitude would be imperceptible; as a matter of fact our data throw no light whatever upon this question.

Inferences from the histology of the dorsal roots. Previous studies have shown that the configuration of the main parts of what we now designate the *A* elevation can be accounted for by the potentials in ordinary myelinated axons. The dorsal roots contain, in addition, a few small finely myelinated fibers and unmyelinated fibers. Whether these form a sufficient fraction of the total area to supply the observed relative area (up to $\frac{1}{3}$ of *A*) of the *C* action potential does not appear likely, though it remains for future work to settle this question.

Excised ventral roots. The results obtained in the case of the ventral

[8] The word trunk, unqualified, will be used to designate this part of the peripheral nerve.

roots varies with the root. Considering first the isolated roots, strong stimulation of the *6th* or *7th roots* invariably elicits, in addition to the *A* elevation, a wave traveling at rates ranging usually between 0.7 and 0.9

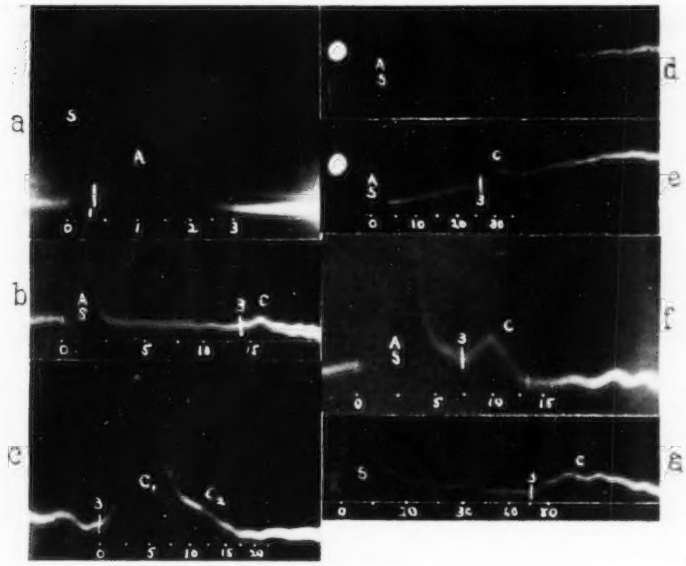

Fig. 11. Ventral root action potentials of the bullfrog. $\times \frac{2}{3}$.

a, 7th root (5/3/29). 4 panels, but greatly shunted to bring record within range of film, 4,000ω. Conduction distance 1.1 cm. Stimulus maximum for *A*. Conduction rate 37.0 m.p.s.

b, same as *a* except that line is slower (24,000ω), and stimulus maximum for *C*. Single transit.

c, same as *b* except that line is still slower (29,000ω), *C* is further forward where the time intervals are longer, and the shunt is removed. Zero of time is at the beginning of *C*. Single transit.

d, 7th root (7/11/29). 4 panels, 180,000 1/2 mf. 28°C. Trunk stimulated (stimulus maximum for *C*) distal to rami, lead from root. Conduction distance 3.8 cm. Shock and *A* elevation merged. Single transit.

e, same as *d* except that trunk is stimulated central to rami. Conduction distance 1.8 cm. *C* rate 0.72 m.p.s. Single transit.

f, same as *d* except that root is stimulated, conduction distance is 0.6 cm., and line is faster (50,000ω). *C* rate 0.83 m.p.s. Note that there is a *C* wave in *e* and *f*, but not in *d*. Single transit on duplitized film.

g, 7th root (7/15/29) stimulated (at maximum for *C*, 5.4 cm.), lead from rami. 233,700ω, 1/2 mf. 30.4°C. Conduction distance 2.0 cm. *C* rate 0.28 m.p.s. In the same preparation there was no deflection when the dorsal root was stimulated. Single transit.

m.p.s. (see fig. 11, *a*, *b*, and *c*). Occasionally the rate is as high as 1.0 or as low as 0.6 m.p.s. Evidently this is a somewhat faster moving elevation than the posterior root *C*. To obtain it in maximum amplitude the coil has to be down to about 5 or 6 cm. Irritability and conduction rate seem to put this wave in the *C* category. Upon the whole the ventral root *C* wave is simpler in form than the posterior root *C*. Usually it seems to be made up of two parts, a high first wave C_1 and a lower second wave C_2 (see fig. 11, *c*). Relative to *A*, it is the highest of all the *C* elevations, sometimes attaining a thirteenth the amplitude of *A*. Its area may be 36 per cent that of *A*.

Over twelve preparations of the *8th ventral root* have been examined. All had magnificent *A* elevations, and, with but one exception, no other elevations. The exception had a very small *C* wave.

In the case of the *9th ventral root*, of twelve preparations examined, all with good *A* elevations, nine exhibited an elevation traveling at rates ranging usually between 1.0 and 0.7 m.p.s., but these were very much lower in amplitude than the *C* waves of the 6th and 7th roots; three had not a trace of any wave other than *A*.

There is no elevation in the motor roots having the characteristics of *B*.

The ventral root C beyond the root. The motor *C* elevation passes in either direction between the root and the trunk, but in a majority of the cases the whole of it leaves the trunk by the rami communicantes. Thus of six of the 7th roots studied the entire elevation left the trunk in four; in two preparations, both from the same animal, a very small wave, started by stimulation of the ventral root and traveling 0.6 m.p.s., was recorded in the trunk beyond the rami. In no other root-trunk preparations has a ventral root *C* wave been found passing the point of emergence of the rami. The number of observations, however, has not been large enough to justify a definite statement at this time regarding any but the 7th preparation.

The result usually obtained in the 7th root-trunk preparation is as follows: upon stimulation of the preparation in successive trials from below upwards while leading from the root, the *C* elevation first appears in the record when the stimulating electrode touches the point of origin of the rami communicantes, as may be seen in figure 11, *d*, *e* and *f*. Direct proof that the *C* fibers leave by the rami can be obtained by recording in the rami the action potential started by stimulation of the trunk close to its origin; a very fine double *C* wave results. Or, if the motor root (7th) is stimulated with shocks gradually increasing in strength and a lead is taken from the corresponding ramus nothing presents in the record excepting the shock artefact until the coil approaches 10, when an elevation traveling at the rate of about 0.3 and 0.6 m.p.s. (two preparations) appears; it attains maximum height at a coil separation of 4 to 5 cm. (see fig. 11, *g*). The *C* elevation is propagated, if anything, more slowly in the trunk and ramus than in the root.

The path taken by the motor *C* elevation and the peculiarity of its distribution between the several roots indicates clearly that it is formed by preganglionic fibers of the sympathetic system (see Langley and Orbeli, 1910).

Inferences from the histology of the white rami and motor roots. The white ramus of the frog is known to be composed mainly of fine myelinated fibers. A teased 7th white ramus of the bullfrog we have found to be made up almost exclusively of thin-sheathed myelinated fibers measuring for the most part 4μ in diameter, but ranging up as high as 7μ even. This picture has been confirmed by osmic acid preparations.

Past work indicates that the potentials of the ordinary myelinated fibers make up the *A* elevation of the anterior root. If the *A* and *C* elevations are produced by distinctive fibers it seemed that it should be possible to recognize the two fiber types by a histological comparison of the 7th and 8th motor roots since the 7th has a well-developed *C* elevation and the 8th none. Accordingly osmic acid preparations were made of roots whose action potentials had been recorded. The histological differences were not nearly as striking as we expected to find them. Both of the roots contain very fine myelinated fibers, the 8th a few, the 7th considerably more, but obviously not enough to make the white ramus. The 7th contains also some undifferentiated ground substance, possibly unmyelinated fibers, but again probably not enough, if they become myelinated, to make up the white ramus. Since usually all, or practically all, of the motor root *C* passes into the ramus, since it passes with equal facility in either direction, since the dorsal root contributes no appreciable potential to the white ramus, and since the ventral root *C* wave is propagated at about the same rate in root, trunk and ramus (slower, if anything, in the trunk and ramus), the histological observations, as far as they go, seem to indicate, 1, that myelinated fibers as large in diameter as 4μ or more can conduct at a *C* (0.7 m.p.s.) rate, and, 2, that with the apparent alteration in the histology of the *C* fibers from that of root fibers, whatever it may be, to that of the fibers of the white rami there is little, if any, alternation in the rate at which the impulse is conducted.

Relative irritabilities of dorsal and ventral root C fibers in the frog. By stimulating the 7th trunk central to its rami and leading from the dorsal and ventral roots separately it becomes possible to determine accurately the relative irritabilities of the dorsal and ventral root *C* fibers. Repeated alternation of the readings between the two roots compensates any error that might arise on account of the necessary succession of the observations. Readings in one preparation, three from each of the roots, gave thresholds of 10, 10, 10 in the ventral root and 8.5, 8.5, 8.5 cm. in the dorsal root. The calibration curve of the induction coil indicates that shocks at 8.5 are about twice as high as shocks at 10 cm. It will be recalled that

the motor *C* wave travels faster by about 80 per cent than the sensory *C* wave; the relative irritabilities of these two sets of fibers agree fairly well, therefore, with their relative conductivities.

Mammalian roots. A study similar to that made in the bullfrog has been carried out on the spinal roots of the cat; action potentials have been recorded in excised dorsal and ventral roots of the 7th lumbar and 1st sacral segments, usually at body temperature; also in the tibial branch of the sciatic nerve either *in situ*, and then with cut ends exposed to room temperature, or excised with a root attached and at body temperature, the stimuli in all cases being applied to one or the other of the roots above mentioned. There were certain technical difficulties more or less peculiar to this set of experiments. 1. The strong induction shocks needed stimulated not only at the cathode but often also at the anode (Heinbecker, 1928); often, too, the responses were repetitive; and when these things happen the resulting pictures were very difficult to interpret. 2. The repeated strong stimulation needed in such instances to make possible the interpretation of the picture soon damaged the preparation. 3. When the preparation was left *in situ* the strong shocks often spread to, and stimulated, muscle. To avoid the possibility of mistaking escaping muscle action potentials for nerve action potentials only those observations were regarded as acceptable during the course of which the muscles did not obviously contract. A brief account of the results of this series of experiments will suffice.

In the *excised dorsal roots* the usual *A* elevation has again been demonstrated (Erlanger, Bishop and Gasser, 1926) and, in addition, an elevation having the conduction rate and relative irritability of *C* of warm-blooded somatic nerve. Our best record of such a wave is seen in figure 12, *a;* it was obtained from a root at 25.5°C., stimulated maximally for *C* (coil at 5.4 cm.). The coil positions for the thresholds of the *A* and *C* elevations were 27.7 and 7.7 cm., respectively. The amplitude of the *A* elevation was not recorded; but on the basis of the amplification factor it can be asserted that it exceeded that of *C* at the least eighteen times. The conduction rate of the first *C* elevation was 0.71 m.p.s., of the second 0.25 m.p.s. In the root at body temperature the rates of the first *C* wave have usually ranged between 1.8 and 1 m.p.s. We have never seen in these roots any wave consistently occupying the position of *B*.

When the root is stimulated and the *lead* taken *from* the *tibial nerve*, slow moving waves are recorded, but, as might be expected, they are low in amplitude. At very short distances of conduction, even, and in the roots themselves, *C* is apt to be low; it is not surprising, therefore, that only low *C* elevations have been recorded at the longer distances of conduction and at the much greater dilutions of the responding *C* fibers by unaffected fibers that obtain when the root is stimulated and the lead taken from the

peripheral nerve. Two records are reproduced, one, figure 12, *b*, from an *in situ* preparation, the other, figure 12, *c*, from a preparation mounted in the moist chamber. Both show a low but definite double elevation the first wave of which travels at the rate of 1.8 m.p.s. in one and 0.85 m.p.s.

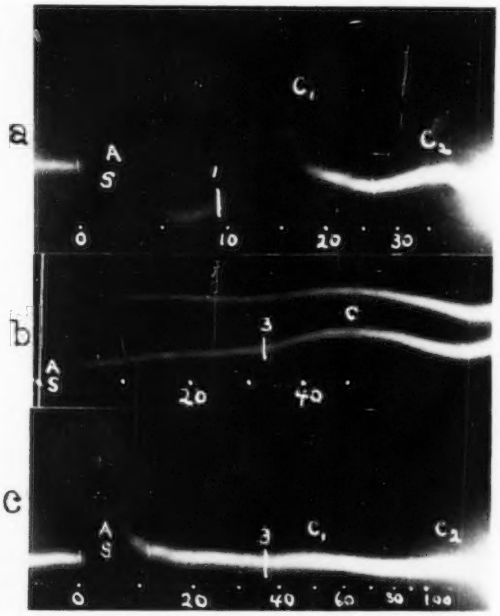

Fig. 12. Dorsal root action potential in the cat. Natural size.

a, 1st sacral (4/26/29) at 25.5°C. 4 panels, 25,000ω. Conduction distance 0.7 cm. Stimulus maximum for *C*, 5.9 cm. *A* merged with shock escape. Rates, C_1 0.71, C_2 0.25 m.p.s. Single transit.

b, two deflections from an *in situ* 1st sacral root-tibial preparation (4/13/29). 4 panels, 139,000ω. Body temperature. Root stimulated, lead from tibial. Conduction distance 10 cm. approximately. Stimulus maximum for *C*, 2.9 cm. *C* rate 1.8 m.p.s. The line is deformed by the shock artefact. Single transits.

c, isolated 1st sacral root-tibial preparation (5/8/29) at 36°C. 4 panels, 69,000ω. Conduction distance 3.15 cm. Stimulus maximum for *C*. Rates, C_1 0.85, C_2 0.35 m.p.s.; *A* (in another record) 52 m.p.s. The thresholds were *A* 29.2, *C* 5.6 cm. Single transit.

in the other, the second elevation (in *c*) at the rate of 0.35 m.p.s. It should be pointed out that when first seen on the face of the oscillograph these *C* waves were considerably higher in amplitude; by the time the records were made they had suffered as a result of the preliminary stimulation. The

thresholds of *A* and *C* in preparation *c* were 29.2 and 5.6 cm., and the conduction rate of *A* 52.2 m.p.s.

Nothing resembling a *B* wave has been seen in these dorsal root preparations.

Inferences from histology. Mammalian dorsal roots have been found by Ranson (1914) to contain more than twice as many unmyelinated fibers as myelinated. He has found, furthermore, that all of the former, as well as a few fine myelinated fibers, pass from the dorsal roots into Lissauer's tract. The obvious inference is that the *C* waves we have observed in mammalian dorsal roots are formed by the action potentials of these fibers. Whether the fine myelinated fibers are present in sufficient number to produce a legible deflection we do not know. If they are, the absence of any slow moving wave other than *C* may be taken to indicate that these fine myelinated fibers and the unmyelinated fibers both conduct at the *C* rate.

CONTRIBUTION OF THE GRAY RAMI TO MIXED NERVE. In the *bullfrog* it is a simple matter, since the parts involved are covered only by peritoneum, to make a preparation consisting of one of the trunks of the lumbosacral plexus, formed by the union of any one of the contributing pairs of spinal roots, with its rami communicantes attached. The lead in these experiments is from the distal end of the preparation from a point somewhat central to its union with the other sciatic components, and the stimuli are applied to the sympathetic chain, often with one ganglion intervening, and to the trunk central to the rami. By including one ganglion a sufficient length of gray ramus is obtained to obviate danger of spread of the stimuli through the short rami to the trunk.

The results obtained have been perfectly definite and can best be presented by citing just one of the experiments (see fig. 13). The record (*a*) resulting from stimulation of the trunk (the 9th in this case) maximally is made up of an *A* elevation traveling 54 m.p.s. and a double *C* elevation, the two parts of which travel 0.78 and 0.45 m.p.s., respectively, rates that are typical of *C* components. These elevations appear on the screen as their characteristic thresholds are reached. *B* is conspicuously absent, as may be seen by comparing this record (*a*) with the next record (*b*).

When the rami are stimulated maximally the record (*b*) consists of two elevations which by their conduction rates, namely, 3.11 and 0.51 m.p.s., (and their thresholds) are definitely identified with *B* and *C*. Then the trunk and the rami are laid across the electrode and together stimulated maximally. The record obtained (*c*) obviously is a combination of records *a* and *b* of this series, and has the appearance of a typical mixed nerve action potential; it is made up of an *A* elevation traveling at the rate of 50.0 m.p.s., a *B* elevation traveling 2.10 m.p.s. and a double *C* elevation with components traveling 0.66 and 0.40 m.p.s. The discrepancies in

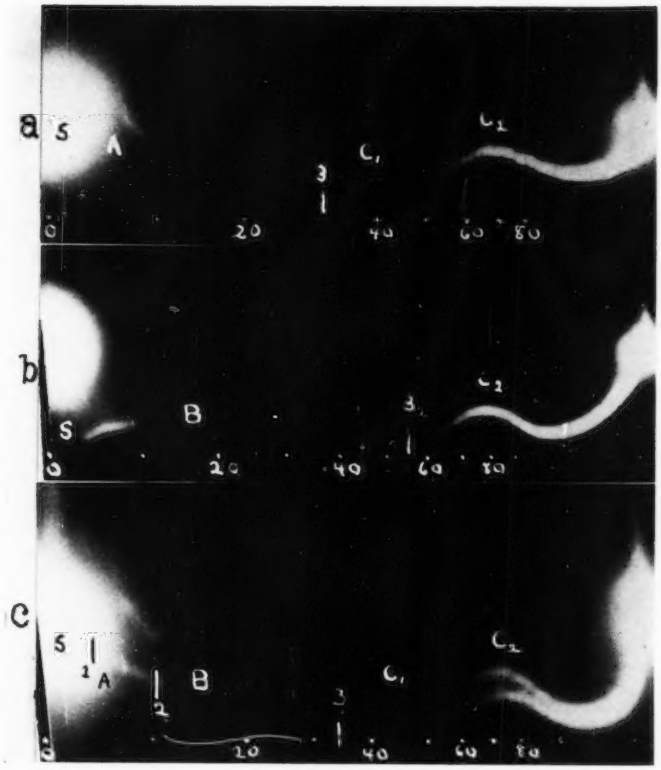

Fig. 13. Records of the gray ramus contribution to mixed nerve in the bullfrog (4/12/29). Preparation consists of the excised 9th trunk with rami attached. The lead in *a*, *b*, and *c* is from the trunk peripheral to rami. 4 panels. All single transits. The records are written on a line deformed by the shock escape. Natural size.

a, stimulus applied to the trunk central to rami. 60,000ω. Conduction distance 2.8 cm. Stimulus maximum for C, 4.4 cm. The record shows two elevations, namely A traveling at an A range of rate, not determinable in this record, and two C elevations, C_1 traveling 0.73 and C_2 0.44 m.p.s.

b, rami stimulated. 50,000ω. Conduction distance 2.25 cm. The record shows two elevations, B traveling 3.11 m.p.s. and C (probably C_2) traveling 0.5 m.p.s.

c, rami and trunk stimulated simultaneously on one stimulator. Two single transits. The record shows an A elevation, rate not determinable here, a B elevation traveling 2.88 m.p.s. and two C elevations, the first, indistinct, moving 0.75 and 0.43 m.p.s., otherwise the same as *b*.

the conduction rates are not beyond the limit of error considering the shortness of the nerve and the differences in the strength of the stimuli.

It should be pointed out that the conduction rates of the *B* elevations, but not of the *C* elevations, in this series of experiments, have been slower than those of the *B* elevation as measured directly in the sciatic nerve, though but little if any slower than *B* in the bullfrog's skin nerve. Whether

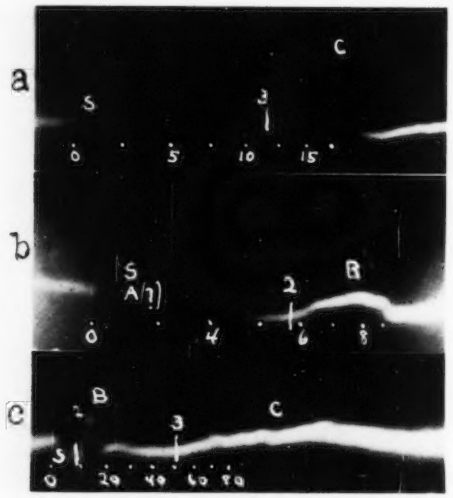

Fig. 14. Records of the gray ramus contribution to mixed nerve in warm-blooded animals. All single transits. Natural size.

a, excised gray ramus preparation of the cat (4/24/29). The gray ramus is stimulated and the lead is from the lumbosacral trunk to which it runs. 4 panels, 24,000ω. 36.5°C. Conduction distance 1.2 cm. Stimulus maximum for *C*. The only elevation is *C*, traveling at the rate of 0.98 m.p.s.

b, record from a gray-ramus-femoral-nerve preparation of the dog, *in situ* (5/15/29). 4 panels, 12,000ω. Rectal temp., 34.5°C. Conduction distance 8.3 cm. Stimulus maximum for *B*. *B* conduction rate 11.9 m.p.s. On duplitized film.

c, same as *b*, but stimulus maximum for *C*, and line slower, (162,000ω). *B* rate 9.3 m.p.s., *C* rate 1.62 m.p.s.

the intervening ganglion on the path of the sympathetic fibers here is responsible for this difference is not the province of this paper to ascertain.

Confirmation of the entrance of *B* by way of the gray ramus can easily be obtained from a preparation consisting only of the trunk by stimulating on either side of its junction point with the rami, while leading peripherally; a *B* wave appears in the record only when the electrodes are at or peripheral to the ramus junction.

Similar experiments to those just described have been carried out in the

cat and *dog*. Gray ramus-nerve preparations have been examined both after excision, but at body temperature, and *in situ*. The excised preparation consists of a segment of nerve trunk 1 to 2 cm. long, cut high enough up in the intervertebral canal to include the rami, and its communicating branches from the sympathetic chain, themselves usually over 1 cm. The stimulus is applied to the ramus and the lead is from the nerve trunk. The *in situ* preparations were made according to the method of Langley (1891). In the anesthetized animal a good exposure is made of the abdominal sympathetic chain. The appropriate part of the chain is divided into segments by transection immediately below each ganglion. The preparation is arranged for stimulation centrifugally, the femoral nerve is cut above the origin of the saphenous nerve, and its freed central end placed upon the leads. The two ends of the preparation, therefore, are at room temperature, the rest at a low body temperature. The exposed nerve is kept moist by frequent applications of Ringer's solution to it, and, by trial stimulations, the gray ramus is found that gives the highest action potential in the nerve.

All of the preparations successfully made (nine) have given good *C* waves (see fig. 14, *a* and *c*); four, in addition, all femoral nerve preparations, have disclosed a wave, with its threshold definitely in the *B* range, traveling at rates that have varied between 17.9 and 8.4 m.p.s. (see fig. 14, *b* and *c*). In the experiment in which the conduction rate was 8.4 m.p.s. (dog) the rate of the *B* wave in the saphenous nerve of the same animal under exactly the same conditions was found to be 8.5 m.p.s. The *B* wave in the gray rami has had in all cases the characteristic brevity of warm-blooded *B*, to which reference will be made in the discussion. Four of the gray ramus preparations displaying a *C* but not a *B* wave were of the sciatic plexus; for some unknown reason we have as yet failed to ascertain the path by which *B* fibers find their way into the sciatic nerve in warm-blooded animals. Whatever the meaning of this experience may be, our experiments have disclosed no other source of *B* fibers than the gray rami.

Discussion. *On fundamental differences between A, B and C fibers.* The first question that suggests itself here has to do with the possibility of recognizing by their morphology the fibers whose action potentials make up the *A*, the *B* and the *C* elevations. In a preliminary report based upon a parallel investigation of fibers in the involuntary nervous system conducted by Heinbecker in this laboratory (1929) the conclusion was reached that "unmyelinated fibers can be identified," among other ways, "by a conduction rate slower than that of the ordinary myelinated fibers of peripheral nerves. . . . . " In a subsequent preliminary report based on the study of nerves of the involuntary nervous system and various other nerves Heinbecker and Bishop (1929) are lead to infer that the *B* elevation corresponds to the thinly myelinated, the *C* elevation to the

unmyelinated axons in islands composed of these fibers. Now, it turns out that there is one set of observations made in the present investigation that seems to be incompatible with this view, namely, those concerned with the *C* elevation in the ventral roots of the bullfrog. This elevation passes into, and seems to account completely for, the white ramus action potential. The white ramus fibers are thinly myelinated ranging in diameter between 2 and 7μ, the 3–4μ fibers predominating; yet they conduct at or very close to the *C* rate and have nearly, if not quite, the irritability of *C* fibers. An observation also of significance in this connection is the apparent lack in the roots of a sufficient number of these small myelinated fibers to make up the white ramus, coupled with the fact that in the root and the white ramus the propagation rates of *C* are substantially alike. Evidently, here are fairly large, though thinly myelinated, fibers that have the conductivity and irritability of *C* fibers; and in the root the continuations of these fibers, apparently altered in histology, conduct at the same rate as the fibers in the white ramus. In view of these findings it seems best for the present not to attempt a classification of the *B* and *C* types of fibers on a histological basis.

It has been shown above that of the action potential area of mixed nerve there is not enough left, after deducting the area of the *A* potential, to provide all of the *B* area. Other sources of potential that remain to be called on are the unmyelinated fibers and perhaps the smaller myelinated fibers, if the area of the potentials of the individual fibers of this variety is larger than that of the *A* variety. Heinbecker (1929) presents evidence indicating that fibers of slow conduction have relatively long lasting action potentials; if, in addition, it could be shown that the amplitude of their potentials is not correspondingly lower the small myelinated fibers might suffice to supply the full amount of the energy that is liberated with the B wave, and in this respect the view would still be tenable that *B* of somatic nerves derives its potential from small myelinated fibers and *C* from unmyelinated fibers. But now that small myelinated fibers have been found that have the conductivity and irritability of *C* fibers, the need of exercising caution in assigning potential waves to types of fibers becomes very obvious. Moreover, in certain animals, the dog's saphenous, for example, we have been unable to distinguish any considerable group of fibers that can be regarded as thinly myelinated, yet the action potential of this nerve exhibits an exceptionally well developed *B* wave.

Another consideration that may have some significance in this connection is the contention of neurologists, generally, that one and the same nerve fiber may in part of its course be myelinated, in another unmyelinated. This seems to be particularly true of postganglionic fibers (see Ranson and Billingsley, 1918); but motor fibers, even, may lose their sheaths, some distance, for instance, from their terminations (Garven, 1925). It would

be surprising if, merely through loss or acquisition of myelin, fibers could be so changed in their reactions as to pass from one of the groups, *A*, *B* or *C*, into another. It seems much more reasonable to suppose, in view of our findings, that the striking differences in conductivity and irritability of the three fiber groups are due to some fundamental difference in the composition of the fibers, related, perhaps, in some way to the systems to which the fibers belong. The *B* fibers, for instance, evidently are postganglionic. However, there are, presumably, other postganglionic fibers; for mixed nerve acquires quite a large *C* component by way of the gray rami. The *A* group is made up of voluntary motor fibers and of sensory fibers, probably those belonging to the epicritic group. The *C* fibers of the dorsal roots would then be left to care for protopathic sensation, a possibility that has been considered by Ranson and Von Hess (1915) in relation to the unmyelinated fibers of the posterior roots. These illustrations are certainly suggestive of the possibility that there is a difference in the materials composing the fibers of each of the groups that accounts for the differences in their reactivities.

The adoption of such a view would by no means preclude the possibility that structural differences also affect conduction and irritability. Within each of the groups the fibers differ among themselves rather widely in these respects. This gradation, in so far as *A* is concerned, has been shown to be referable to the diameters of the *A* fibers,—the larger the fiber the higher its conductivity and irritability (Gasser and Erlanger, 1927). And it may well be that diameter differences of the constituent fibers account for the range in conductivity and irritability within the other groups, *B* and *C*, also.

On the relation of A, B and C of cold-blooded nerve to A, B and C of warm-blooded nerve. In so far as concerns their origins, the evidence is quite clear that the *A*, *B* and *C* fibers of cold-blooded animals are the homologues of the *A*, *B* and *C* fibers of warm-blooded animals; the corresponding groups obviously are alike in respect to kind. Nevertheless it is possible to demonstrate in the case of one of the groups, namely, *B*, certain quantitative differences related to the class of animals, warm- or cold-blooded, to which the fibers belong.

We may consider first differences in conduction rate. Since conduction rate depends upon fiber diameter (among the large myelinated fibers, at least) and since the *B* and the *C* fibers have not yet been definitely identified, a comparison of the ratios of *A* rates, of *B* rates and *C* rates, warm-blooded to cold-blooded, can for the present have only a tentative value in this connection. Nevertheless, let us assume that the relative differences in diameters of the largest fibers in each of the groups, *A*, *B* and *C*, of warm- and cold-blooded animals are the same and that temperature affects all of the fibers alike. The justifiability of the latter assumption has already been demonstrated as regards *A* (Gasser and Erlanger, 1927);

and the fact, shown above, that at room temperature *C* of warm-blooded nerve has about the same conduction rate as *C* of cold-blooded nerve indicates the applicability of the rule to this process, also. Then, from average conduction rates numerical values can be derived expressing the conduction rates of the *A* elevations, the *B* elevations and the *C* elevations in nerves of one class of animal relative to those in nerves of the other class. The number of observations in the dog (saphenous) and in the bullfrog (sciatic) is sufficient to make a comparison of this kind worth while. The values obtained in these animals are

$$\frac{\text{Dog A}}{\text{Bullfrog A}} = \frac{82.8}{42.0} = 1.97$$

$$\frac{\text{Dog B}}{\text{Bullfrog B}} = \frac{13.8}{4.5} = 3.07$$

$$\frac{\text{Dog C}}{\text{Bullfrog C}} = \frac{0.97}{0.59} = 1.6$$

The *A* and the *C* ratios, it is seen, do not vary so very widely from a mean of 1.80, a result which may be taken to indicate that the *A* and the *C* fibers of the dog and frog have comparable conduction rates. The very much larger ratio derived from the comparison of the *B* conduction rates suggests either that the *B* fibers of the two classes of animals differ more widely either in respect to size or in respect to some other structural factor affecting conduction rate, or in constitution than do the *A* or *C* fibers. The *B* fibers of the dog, furthermore, seem to be somewhat more irritable relative to *A* and *C* than those of the bullfrog, though our data relative to this property are not sufficiently numerous to justify more than a tentative statement regarding it. Differences in constitution, if they obtain, might be regarded as a reason for suspecting that the *B* fibers of the two classes of animals may not be comparable functionally. Opposed to any functional difference, however, is the fact that in both mammals and amphibia the *B* wave gets into peripheral nerves apparently only by way of the gray rami.

The *B* waves of cold- and warm-blooded nerve differ from each other in still another respect, namely, as regards their durations, both absolute, and relative to the durations of their companion *A* and *C* waves. The data for making these comparisons are derived from table 1, and include all of the observations on the sciatic of the bullfrog and the dog's saphenous that are sufficiently complete for the purpose. They are collected in Table 4. The comparison is facilitated by the fact that the average conduction distances turn out to be alike for the two nerves.

The table shows that if we multiply the average durations of the eleva-

tions in warm-blooded nerve by three, which in this case seems to be the factor necessary to care for the effect of the difference in temperature at which the two sets of observations were made, the *A* values and the *C* values derived agree remarkably closely among themselves. The *B* values, however, differ widely, *B* in the cold-blooded nerve being almost five times as long as *B* in the warm-blooded nerve.

The brevity of warm-blooded *B* signifies, and to this reference has already been made, that it is constructed of axon action potentials traveling at rates that range between relatively narrow limits. In addition, it means that the component action potentials must have a duration that is less

TABLE 4

	NUMBER	CONDUCTION DISTANCE	DURATIONS					
			Absolute in σ			Relative		
			A	B	C	A	B	C
Bullfrog sciatic		5.8	3.60	22.35	68.0	1	6.2	19.0
	8	7.4	5.67	33.55	146.0	1	6.0	25.7
	9	9.1	5.31	32.65	150.0	1	6.1	28.3
	Average	7.4	4.86	29.52	121.0	1	6.1	24.0
Dog saphenous	12	9.00	1.60	3.80		1	2.00	
	14	4.40	1.06	1.96		1	1.85	
	16a	12.00	2.51	2.53	34.4	1	1.00	13.7
	16b	9.00	2.20	1.90	30.3	1	0.90	13.7
	17a	5.90	1.38	1.43	58.4	1	1.04	42.3
	17b	5.90	1.36	1.75		1	1.30	
	18	6.95	1.16	1.66	59.2	1	1.43	49.8
	Average	7.6	1.61	2.15	45.6	1	1.2	29.9
	× 3		4.83	6.45	136.8			

than 1.2 σ, less, indeed, than the duration of the briefest of the *B* elevations, which is 0.9 σ. Since the duration of the *A* axon action potential lies in the vicinity of 0.6 σ (Gasser and Erlanger, 1927), it follows that the action potentials of the *A* and the *B* axons of warm-blooded nerve are of the same order of magnitude. We have no data bearing on the duration of the action potential in the *B* fibers of cold-blooded nerve; we assume, however, merely on the basis of duration of the *B* elevation, that it is considerably longer than that of the corresponding *A* fibers. The *B* fibers of warm-blooded nerve, therefore, differ from those of cold-blooded nerve (1) in conducting relatively more rapidly (2) in being slightly more irritable relatively and (3) in having a relatively brief action potential.

SUMMARY

On the basis of conductivity and irritability it is possible to distinguish in all somatic nerves three groups of fibers, designated *A*, *B* and *C*. The *A* fibers are those that produce the familiar somatic nerve action potential in which the cathode ray oscillograph has disclosed *alpha*, *beta* and *gamma* waves; they include the large myelinated fibers down to about 5μ. The *B* and the *C* fibers include the myelinated fibers not contributing to *A* and unmyelinated fibers.

In round numbers the range of conduction rates of the fibers contributing to *A* in the dog and cat is 90 to 30 m.p.s., of the *B* fibers 20 to 10 m.p.s.,

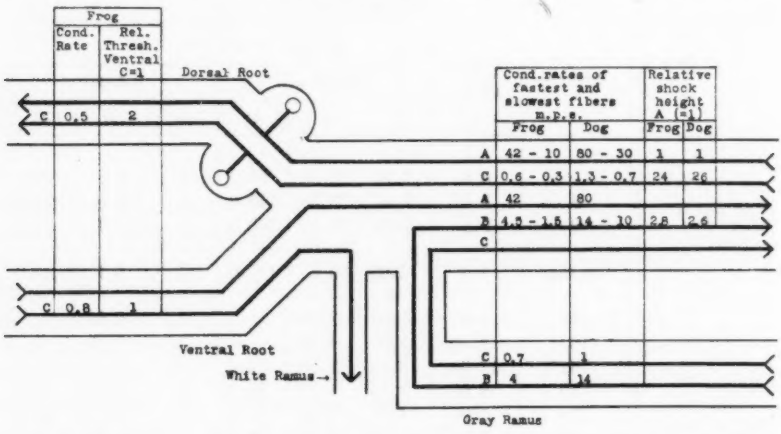

Fig. 15. Diagram indicating the sources and a typical set of reactivities of the fiber groups that contribute to the action potentials of mixed nerve and spinal roots.

of the *C* fibers 1.6 to 0.3 m.p.s.; in the bullfrog the ranges are, for *A*, 50 to 10 m.p.s., for *B*, 5.5 to 1.3 m.p.s., and, for *C*, 0.9 to 0.2 m.p.s. In the green frog the rates are all somewhat slower than in the bullfrog.

The differences in irritability of the three types of fibers are such that by gradually increasing the strength of stimulus (induction shocks) it is possible to elicit in succession, with intervening gaps, the *A* elevation of the action potential, the *B* elevation and the *C* elevation. With the inductorium used by us under a more or less constant set of conditions the coil separations in centimeters in round numbers have been for the *A* threshold and maximum 30 and 20, for *B*, 18 and 10, and for *C*, 8 and 4. Taking the shock height at 30 cm. as 1 the approximate shock heights at the other coil positions just given are 2.1, 2.4 and 6.0, 15.7 and 203.0, respectively.

The *A* elevation always is compound, *B* occasionally so and *C* usually. In the sciatic of the bullfrog at the usual distances of conduction the amplitudes of the elevations relative to *C* (= 1) in round numbers typically are 5 and 100 for *B* and *A*, respectively; in the skin nerve of the bullfrog the relative heights may be 1, 3 and 40 and in the saphenous of the dog (a skin nerve) 1, 4 and 40 for *C*, *B* and *A*, respectively.

The *B* fibers of warm-blooded nerve have a somewhat higher reactivity relative to *A* and *C* than those of cold-blooded nerve. When allowance is made for difference in temperature the *A* fibers in both warm- and cold-blooded nerve conduct at about the same rate, likewise the *C* fibers, but the warm-blooded *B* fibers conduct considerably more rapidly than the cold-blooded *B* fibers. Similarly, the durations of the cold-blooded and warm-blooded *A* and *C* waves are each of the same order of magnitude; but the warm-blooded *B* elevation has about a fifth the duration of cold-blooded *B*. Its duration is such as to indicate that in warm-blooded nerve the duration of the axon action potential of *B* is of the same order of magnitude as that of *A*.

The *A* fibers enter somatic nerves via the spinal roots, *B* fibers via the gray rami and *C* fibers via the dorsal roots and the gray rami in both warm- and cold-blooded animals; but, seemingly, in warm-blooded animals not all of the gray rami transmit *B* fibers.

In the frog, the 6th, the 7th and the 9th (usually), but not the 8th (with rare exceptions), ventral roots, these being the only roots investigated, contain fibers which produce a *C* elevation traveling at the rate of 0.8 m.p.s.; these fibers leave the nerve by the white rami, though occasionally (observed twice in the 7th root) some of them pass on in the peripheral nerve. Since the fibers of the white rami are for the most part thinly myelinated and range around a diameter of about 4μ, it follows that such fibers, despite their relatively large size, can conduct at a *C* rate. Undoubtedly, unmyelinated fibers also conduct at the *C* rate; but it has not yet been precluded that they, also, may conduct at a *B* rate. For the present, therefore, it is manifestly impossible to define *B* and *C* fibers on a histological basis.

Figure 15 summarizes schematically the main findings of this research.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

XIII. The Relation of Gastric Motility to Anhydremia in Vitamin B-Deficient Dogs[1]

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Recent studies on the physiology of the stomach in vitamin B-deficient dogs revealed the fact that profound disturbances in the motor function of this organ, nay, even gastric atony, may occur long before neuromuscular symptoms develop (Rose, Stucky and Cowgill, 1930). The constancy with which normal hunger contractions were reëstablished by vitamin B[3] therapy suggested the possibility that the causative factor involved physico-chemical disturbances in the blood—a systemic derangement—rather than actual morbid changes in the stomach itself. This viewpoint was supported 1, by the well-known fact that numerous physico-chemical reactions are readily reversible, and 2, by the demonstration by one of us (R)[4] that severe neuromuscular manifestations of vitamin B deficiency in dogs can be relieved, temporarily at least, within a few minutes by injection of Ringer-Locke fluid into the convulsive animal.

The occurrence of anhydremia in nursling young of the albino rat suffering from B-avitaminosis was reported by Sure and associates (1928). A similar effect on blood concentration in adult animals (rats and dogs) subsisting on a diet deficient with respect to vitamin B was likewise observed (Rose and Stucky, 1928; Stucky and Rose, 1929). It is generally appreciated by biologists that body fluids and organs are intimately related and to a large extent interdependent. The above considerations prompted us to determine whether any relationship exists between disturbances in gastric motility and anhydremia in vitamin B-deficient dogs.

Technique. Apparently normal dogs in good nutritive state were fed

[1] The data in this paper are taken from a dissertation submitted by William B. Rose in partial fulfillment of the requirement for the degree of Doctor of Philosophy, Yale University, 1928.

[2] Porter Fellow of the American Physiological Society, 1926–27.

[3] The term *vitamin B* used in this paper refers to the combination of two or more factors which have been shown to make up this substance (Smith, 1928).

[4] Unpublished data.

a mixture of isolated food substances, diet III (Cowgill, 1923), complete in every known respect except vitamin B. Cod liver oil was administered separately from time to time to doubly insure an adequate supply of fat-soluble vitamins. The diet was supplemented by vitavose[5] as a source of vitamin B during a preliminary observation period. In addition, vitamin powder (Harris)[6] and tomato juice were occasionally used during recovery from B-avitaminosis.

Eight dogs were used. These animals were carefully paired, attention being paid to age, weight, stature and general activity. Four dogs were fed the basal vitamin B-deficient ration; the remaining animals were used as controls. Each control dog was given the same amount of food and water per kilo of body weight as the corresponding vitamin B-deficient animal consumed. In addition, each control received its full requirement of vitamin B regardless of its daily food allotment. In this way the

TABLE 1
First pair

	B-DEFICIENT—DOG 25	CONTROL—DOG 21
	per cent	*per cent*
Increment in hemoglobin	17	21
Increment in blood solids	0.5	2.2
Loss of body weight	27.9	22.8
Changes in gastric motility	Marked decrease	Marked decrease

inanition factor, so dominant in vitamin B studies, was controlled experimentally.

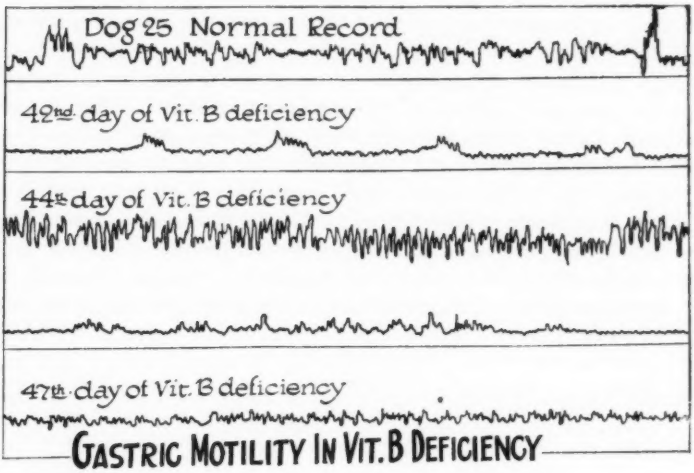
The method for recording gastric hunger contractions is described in a recent communication (Rose, Stucky and Cowgill, 1930).

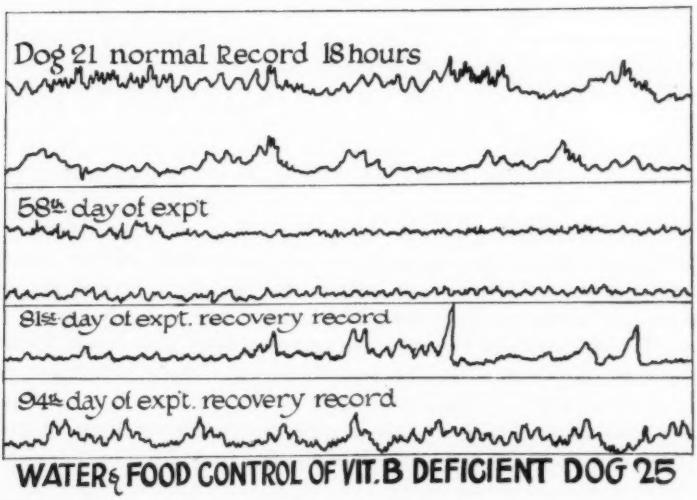
Blood analyses. Blood was obtained by cardiac puncture under fasting conditions. Hemoglobin was estimated colorimetrically by the Cohen and Smith techniqe (1919). Percentages are expressed on the Haldane scale. Blood solids were determined by pipetting approximately one cubic centimeter of blood into an aluminum dish, weighing and drying to constant weight in an electric oven at 105°C.

EXPERIMENTS. It is obvious, when studying a living organism, that we are dealing with a very complex and intricate system, the operation of which involves variables many of which are beyond present experimental control. Hence, it may be expected that the results obtained will not be precisely the same in every case. In the experiments here reported this

[5] Obtained through the courtesy of E. R. Squibb & Sons, New York, N. Y.
[6] From the Harris Laboratories, Inc., Tuckahoe, N. Y.

is indeed the case. Nevertheless, when the data from each pair of animals are carefully analyzed, certain findings become apparent. For this reason the data from each pair of dogs are summarized and briefly discussed.


Dog 25 Normal Record
42nd day of Vit. B deficiency
44th day of Vit. B deficiency
47th day of Vit. B deficiency
GASTRIC MOTILITY IN VIT. B DEFICIENCY


Dog 21 normal Record 18 hours
58th day of expt
81st day of expt. recovery record
94th day of expt. recovery record
WATER & FOOD CONTROL OF VIT. B DEFICIENT DOG 25

Charts 1 (upper) and 2. In these charts are presented portions of the gastric tracings obtained with vitamin B-deficient dog 25 and its water-and-food control companion, dog 21. The records here reproduced are approximately one-seventh the original size.

As regards the 1st pair, the deficient dog fasted voluntarily for 21 days; consequently the control animal did not receive any food except the vitamin-containing supplement during this period. Both dogs manifested an increase in blood concentration as indicated by changes in hemoglobin and total blood solids; both dogs developed a progressive decrease in the vigor, amplitude and frequency of the hunger contractions (charts 1 and 2), although the control animal received an adequate supply of vitamin B daily. It appears, therefore, that the change in gastric motility occurring during the course of vitamin B deficiency is not due directly to the absence of this vitamin from the diet. Apparently, the increase in blood concentration occurring in the control dog 21 was due, in part at least, to the limitation of its water intake to that of the B-deficient companion dog 25. The control animal remained very active throughout, whereas the deficient dog became rather quiet and eventually developed characteristic convulsions and died. This great difference in general activity may partly

TABLE 2

Second pair

	B-DEFICIENT—DOG 26	CONTROL—DOG 22
	per cent	*per cent*
Increment in hemoglobin	0	10
Increment in blood solids	0.4	1.0
Loss of body weight	24.1	2.4
Decrease in hemoglobin after realimentation	21	10
Changes in gastric motility	Almost complete gastric atony	Marked decrease

account for the slightly greater blood concentration found in the control dog.

These results suggest that the cause of decrease in hunger contractions in the control dog 21 may be sought in the anhydremia which occurred. In an attempt to throw further light on this question, experiments were performed on dogs in which water deprivation was the only known variable. The anhydremia that ensued *was associated with complete gastric atony in all cases with a rapid return of vigorous hunger contractions when water was administered* (Rose and Stucky, 1928b).

From the above considerations it appears that the decrease in gastric motility occurring in B-avitaminosis is due in part at least to the concomitant anhydremia that develops.

The data from the 2nd pair seem to invalidate the hypothesis suggested above in the discussion of table 1, namely, that the anhydremia occurring in B-avitaminosis is at least partly responsible for the disturbances in gastric motility observed. Thus, during the course of the vitamin B-de-

ficient period the control dog 22 developed an increase in hemoglobin and total blood solids, whereas the deficient animal apparently failed to show any significant changes in blood concentration. Nevertheless, there was a greater disturbance of gastric motility in the vitamin-B-deficient dog. The latter actually developed gastric atony in the very advanced stages of this deficiency disease, whereas the control animal merely showed a lack of normal sequence of hunger contractions and a distinctly abnormal prolongation of the quiescent period but no gastric atony.

However, the fact that the control dog manifested a greater economy in the utilization of foodstuffs inasmuch as it lost only 2.4 per cent of its initial body weight in contrast to its B-deficient companion which lost approximately one-fourth of its original body weight (24.1 per cent) merits consideration. According to McCarrison (1921) and others that might be mentioned, there is a general atrophy of all body tissues, suprarenals excepted, in B-avitaminosis. It should be stated that the diets used by these other investigators were deficient in other respects besides vitamin B. However, in the present study a much greater loss of body weight (average of 21.2 per cent) occurred in the B-deficient dogs as compared with their food-and-water-control companions (average 5.9 per cent). This finding, in a measure, corroborates the results of McCarrison and others just cited.

In studying any condition associated with generalized tissue atrophy, the blood should be given due consideration. The tendency to an increase in hemoglobin and total blood solids—evidence of blood concentration—might be counter-balanced by the simultaneous partial "atrophy" of the blood as a tissue. This might consist in a decrease in the capacity for blood regeneration in the organism so that repair cannot keep pace with "wear and tear."

If this assumption is correct, and anhydremia was really present in B-deficient dog 26, then this animal should have revealed a definite reduction in hemoglobin and blood solids soon after realimentation with an adequate diet. Such was indeed the case. The hemoglobin dropped 21 per cent and blood solids fell from 20.8 to 17.4 per cent within five days after vitamin B therapy. It now becomes clear why this animal showed gastric atony during the course of the deficiency régime, yet failed to show any significant changes in hemoglobin and total blood solids. On the other hand, the control dog 22 showed a rise of hemoglobin of 10 per cent during the period of inadequate supply of food and water, a relatively mild degree of disturbance in gastric motility and a relatively small decrease in hemoglobin—10 per cent—during the first nine days of recovery. Again we see an apparent relationship existing between blood concentration and gastric motility. This relationship appears to apply with equal significance to the control as well as to the vitamin B-deficient animal.

In the third pair, the vitamin B-deficient dog 27 revealed a slight decrease in blood solids and no significant change in the hemoglobin value—in short, no apparent evidence of anhydremia whatsoever. Yet, tracings of the motility of the empty stomach extending over more than 50 hours showed that gastric atony was complete. This appears to cast doubt on the hypothesis that any relationship exists between anhydremia and loss of hunger contractions. However, in this case as with B-deficient dog 26 (table 2), it is suggested that the anhydremia was "masked" by the lack of adequate regeneration of blood proteins during the course of the deficiency régime. This animal lost 15.7 per cent of its initial body weight. After vitamin B therapy, the hemoglobin value *dropped 38 per cent* and the blood solids showed a corresponding decrease. Unfortunately, this case is complicated because the animal developed an abscess (B. Welchii)[7] a few days after the subcutaneous injection of a vitamin B concentrate. Nevertheless, it is difficult to escape the conclusion that this decrease in

TABLE 3

Third pair

	B-DEFICIENT—DOG 27	CONTROL—DOG 23
Increment in hemoglobin	No change	7 per cent
Change in blood solids	1.5 per cent decrease	No change
Loss of body weight	15.7 per cent	2.2 per cent
Changes in gastric motility	Gastric atony	Slight decrease

hemoglobin value was partly due to the anhydremia that existed because all other uncomplicated cases of vitamin B deficiency in our experience have shown a pronounced decrease in blood concentration after the administration of vitamin B (Rose and Stucky, 1928a; Stucky and Rose, 1929).

In the case of control dog 23 it is of interest to point out that, in spite of enforced starvation for 18 days, this dog still showed some vigorous hunger contractions. This was associated with a small increase in hemoglobin, and one of a series of gastric records showed a long quiescent period, but none of the remaining records of gastric motility manifested any serious changes. Thus it appears that anhydremia and loss of hunger contractions run parallel, although solely on the strength of evidence here presented, one cannot say that they are necessarily interdependent. A separate investigation of this problem on normal dogs, however, indicates that such is the case (Rose and Stucky, 1928b).

[7] The same thing happened on another occasion. This apparently lowered resistance to infection with the bacillus of Welch in B-avitaminotic dogs was made the subject of a separate investigation (Rose, 1928).

On the 47th day of the experiment, vitamin B-deficient dog 28 (chart 3) showed a disturbance in gastric motility as evidenced by a decrease in the vigor of the hunger contractions; the blood at this time revealed a slight increase in solids. On the 62nd day of vitamin B deficiency, there was a marked decrease in gastric motility and a further increase in blood solids. Four days later the animal developed paresis. No gastric records were taken on dogs that exhibited any physical disability.

The control dog (chart 4) did not manifest any changes in the blood throughout the experimental period and gastric motility was just as vigorous at the end as at the beginning of the experiment, although the last record showed some decrease in total activity of the gastric musculature. It is of interest to note that there actually was an increase of 0.3 per cent in blood solids. This is negligible. During the first day of realimentation, however, this dog drank approximately double its usual water intake. Hence, it would appear that this animal was on the verge of developing

TABLE 4

Fourth pair

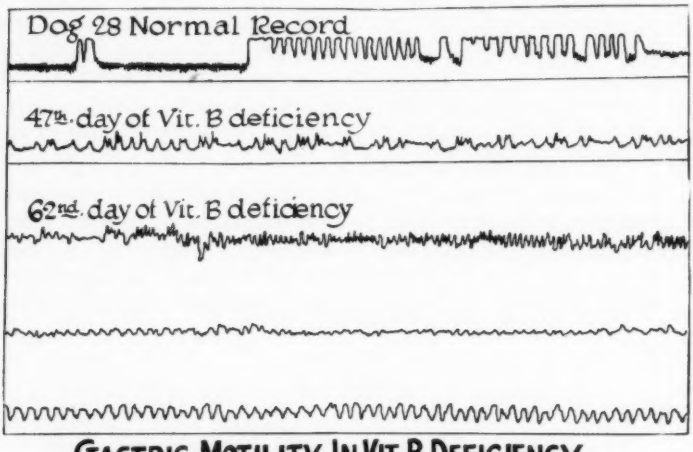
	B-DEFICIENT—DOG 28	CONTROL—DOG 24
Increment in hemoglobin	Data incomplete	No change
Increment in blood solids	2.8 per cent	No change
Loss of body weight	18.9 per cent	No change
Gastric motility	Decreased	No significant change

some degree of anhydremia; this may possibly account for the decrease in total activity of the empty stomach.

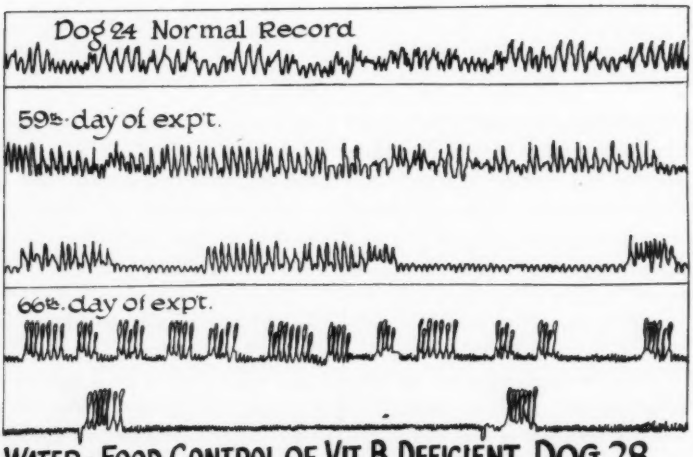
Recapitulation. The data yielded by the first pair of animals show that there was a decrease in gastric motility to approximately the same extent in both the vitamin-deficient dog and its control; that the degree of anhydremia was approximately the same in both cases; that this anhydremia developed in spite of the fact that the control dog received its full quota of vitamin B daily. The results obtained in the second and third sets indicate that the anhydremia, rendered apparent by vitamin B therapy, was much more pronounced in the B-deficient animals than in their corresponding controls, and that the disturbances in gastric motility were likewise much more marked in the vitamin-deficient dogs. The fourth set shows that there was some impairment of gastric motility in the vitamin-deficient dog and some evidence of anhydremia. On the other hand, the control failed to manifest any significant change in blood concentration or gastric motility.

Consequently, then, every case which developed disturbances in gastric

motility during the course of vitamin B deficiency was associated either with a definite increase in hemoglobin and total blood solids or a marked reduction in these constituents of the blood soon after vitamin B therapy



GASTRIC MOTILITY IN VIT. B DEFICIENCY



WATER & FOOD CONTROL OF VIT. B DEFICIENT DOG 28

Charts 3 (upper) and 4. Whereas the vitamin B-deficient dog 28 developed marked depression of gastric motility associated with an increase in blood solids and great loss in body weight, the control animal (no. 24) showed no change in blood solids and body weight and no significant change in gastric motility.

was instituted. This tendency to anhydremia, whether occurring in a vitamin B-deficient animal or its control, ran more or less parallel with a decrease in gastric hunger contractions.

Further experiments were performed to test the hypothesis that a relationship exists between anhydremia and disturbances in gastric motility. Dogs, apparently normal, were deprived of water for six days. They developed complete gastric atony; this atony ran more or less parallel with the anhydremia that ensued. Administration of fluids produced a rapid and remarkable return of vigorous hunger contractions (Rose and Stucky, 1928).

Reinforced by this evidence it appears safe to conclude that an increase in blood concentration resulting either from restricted water intake or vitamin B-deficiency diminished the motor activity of the stomach. Thus, at least one indirect path of influence of vitamin B-deficiency on gastric motility has been brought to light.

Anhydremia is a systemic disorder, hence it is difficult to believe that its inhibitory influence on the myogenic function of the stomach is limited to this organ. It is not inconceivable that the decrease in general muscular activity in vitamin B deficiency may partly be due to this factor. Gross (1924) and Plummer (1927) have reported a decrease in intestinal motility in rats subsisting on vitamin B-deficient rations.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

XVIII. The Effect of Removal of the Liver on the Formation of Ammonia

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The amount of ammonia in the urine is usually considered as an index of the acid-base equilibrium of the body since it is greater after the ingestion of acid and less after the ingestion of alkalis. The formation of ammonia to neutralize acids excreted by the kidney, therefore, furnishes a mechanism whereby the organism may conserve its inorganic bases. The site of the formation of ammonia has been variously ascribed to the kidney, liver, gastro-intestinal tract and to all the tissues of the body. An increase in the amount of ammonia excreted has been shown to be accompanied by a corresponding decrease in the urea in the urine, so that it is evident that ammonia arises from urea or its precursors. It may also be considered that the ammonia excreted is that which has arisen from the deaminization of amino acids and has been combined with acid radicles without the intervening conversion to urea. Since the liver has been shown to be responsible for both deaminization (3) and the formation of urea, (2) the liver might also be expected to have some influence on the excretion of ammonia. With this point in mind we have observed the amount of ammonia in the urine, blood, and tissues of animals after the liver has been completely removed.

A full historical summary of the literature concerning the origin and excretion of ammonia is obviously too voluminous to be included in a paper devoted to experimental work. A comprehensive review of the literature concerning the relation of the liver to ammonia formation has been given by Mann (1927). The recent articles of Gottlieb (1928), Cholopoff (1928), and Bliss (1928, 1929) contain discussions of the problems concerning the formation of ammonia.

Method of experiments. The method of removing the liver has been fully described in previous articles (Mann, 1921; Mann and Magath, 1921). The important considerations are: 1, preparation for surgical procedures to insure sufficient circulation, both systemic and portal, after

removal of the liver so that no other organ or tissue need be interfered with; 2, the liver is removed in one piece and no trace of hepatic tissue remains in the animal, and 3, the operation is performed rapidly with little loss of blood so that recovery from the procedure is rapid, and the animal appears normal shortly after the operation is completed. Without further procedure the animal remains in good condition for from three to ten hours following total hepatectomy and by the administration of glucose in amounts sufficient to maintain the normal level of sugar in the blood, dogs are easily maintained in a normal appearing condition, as in these experiments, for more than twenty-four hours after complete removal of the liver.

Specimens of blood were obtained with a dry syringe from the jugular vein and mixed with powdered oxalate in dry tubes. Specimens of urine were collected in metabolism cages after preliminary catheterization and washing of the bladder. When the urine had been collected the bladder was again catheterized and washed. Since most of the collections were made at short intervals only an occasional specimen was obtained from the metabolism cage.

The ammonia in the blood was determined immediately by the method of Nash and Benedict (1921), the urea nitrogen by the method of Van Slyke and Cullen (1914) and Folin (1922), amino acid nitrogen of the blood by the method of Folin (1922), and the non-protein nitrogen by the method of Folin and Wu (1922) (10). The carbon dioxide combining power of the blood was determined by the method of Van Slyke (1917). The ammonia in the urine was determined immediately by the Folin-Farmer procedure (1922) (10), the urea content by the method of Van Slyke and Cullen (1914), the amino acid content by the method of Folin (1922), and the total nitrogen by the Kjeldahl procedure. The hydrogen-ion concentration of the urine was determined by the colorimetric method and by use of the quinhydrone electrode.

For the determination of ammonia, urea, and amino acid in the tissues, methods similar to those for analysis of the blood were used. Samples of tissue were weighed, then frozen immediately with carbon dioxide, and shaved into a mortar containing sharp sand. The tissues were ground to a pulp and shaken with water before dilution to a definite volume. The solution thus obtained was treated as blood is treated for the various determinations; not more than five minutes elapsed between the time of removal of the tissue from the animal and the completion of the solution for immediate analysis.

Results of experiments. After hepatectomy the excretion of ammonia in the urine is dependent on the several factors which influence the excretion of ammonia in the normal animal. If the volume of urine passed after hepatectomy is within normal limits the ammonia in the urine is also

TABLE 1

*Changes in urea and ammonia in the blood and urine after ether anesthesia injections of glucose and the administration of ammonium chloride (dog 1, weighing 16 kgm.)**

DATE	TIME	COMMENT	BLOOD			URINE						
			Carbon dioxide combining power	Urea nitrogen and ammonia nitrogen	Ammonia nitrogen	Volume	Hydrogen-ion concentration	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Ammonia nitrogen each hour	Urea nitrogen each hour
	a.m.		*volumes per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
8-3-25	8:25	Animal fasted 48 hours; ready for removal of liver (reversed Eck fistula and ligation of portal vein); blood specimen taken; bladder emptied	54.8	6.71	0.01							
	8:30–9:15	Animal etherized										
	9:25	Injected 0.25 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride										
	10:30	Injected 0.25 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride										
	11:25	Blood specimen taken, urine catheterized; injected glucose	53.7	9.20	0.01	35	6.3	555	370	25.6	8.5	123
	p.m.											
	12:30	Injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride										
	1:30	Injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride										
	2:25	Blood specimen taken, urine catheterized; injected glucose + 1 cc. for each kgm. 10% NH_4Cl	51.9	8.05	0.01	90	7.4	1005	813	26.4	8.8	271

	3:30	Injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride									
	4:30	Injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride									
	5:25	Blood specimen taken; urine catheterized	48.1	9.40	0.02	200	7.8	1344	900	92.0	30.7	300
	6:30	Injected 0.5 gm. glucose in 50 cc. physiologic solution sodium chloride									
	7:30	Injected 0.5 gm. glucose in 50 cc. physiologic solution sodium chloride									
	8:25	Blood specimen taken; urine catheterized	53.9	6.71	0.1	250	7.7	1750	1246	54.0	18.0	415
		Total for twelve-hour period				575		4654	3329	198.0	16.5	277

* Four hundred sixteen milligrams of ammonia nitrogen were injected. This gave rise to a definite increase in the urea of the urine and about 90 mgm. of ammonia nitrogen in the urine more than would be anticipated without the injection.

TABLE 2

*Changes in urea and ammonia in the blood and urine after hepatectomy, injections of glucose and the administration of ammonium chloride (dog 1, weighing 16 kgm.)**

DATE	TIME	COMMENT	BLOOD			URINE						
			Carbon dioxide combining power	Urea nitrogen and ammonia nitrogen	Ammonia nitrogen	Volume	Hydrogen-ion concentration	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Ammonia nitrogen each hour	Urea nitrogen each hour
	a.m.		*volumes per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
8-6-25	8:30	Animal fasted 18 hours; ready for removal of liver; blood specimen taken; bladder emptied	53.6	5.68	0.02							
	8:40–9:15	Operation; liver removed at 9:00										
	9:15	Blood specimen taken; injected 0.25 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride		5.95								
	11:30	Blood specimen taken; urine catheterized; injected glucose	53.6	5.15	0.02	70	6.8	448	294	42.0	14.0	98
	p.m.											
	12:25	Injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride										
	1:30	Injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride										
	2:30	Blood specimen taken; urine catheterized; injected 1 cc. for each kgm. of 10% NH_4Cl	49.7	3.06	0.08	170	7.0	388	207	98.6	32.9	69
	3:30	Injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride										
	4:30	Injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride										

5:30	Blood specimen taken; urine catheterized; injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride	43.9	2.00	0.41	160	7.0	256	32	144.0	48.0	11
6:50	Injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride										
8:00	Injected 0.5 gm. glucose for each kgm. in 50 cc. physiologic solution sodium chloride										
8:30	Blood specimen taken; urine catheterized	40.4	1.90	0.28	260	6.9	338	26	91.0	30.0	9
	Total for twelve-hour period				660		1430	559	375.6	31.3	46.6

* Four hundred sixteen milligrams of ammonia nitrogen were injected. This did not influence the diminishing urea of the blood and urine but about 200 grams of ammonia nitrogen appeared in the urine in excess of the amount anticipated without injection. The amount of urea in the urine following hepatectomy is entirely accounted for by the washing out of the urea of the blood and tissues.

found to be comparable to that in a normal animal under similar conditions. Immediately after operation (ether anesthesia) there is a well marked increase of ammonia in the urine which is accompanied by a decrease in the carbon dioxide combining power of the blood and a decrease in the

TABLE 3

*Effect of intravenous administration of alkali after removal of the liver (dog 2, weighing 11.6 kgm.)**

DATE	TIME	COMMENT	BLOOD			URINE					
			Carbon dioxide combining power	Urea nitrogen and ammonia nitrogen	Ammonia nitrogen	Volume	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Ammonia nitrogen each hour	Urea nitrogen each hour
	a.m.		*volumes per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
4-5-28	8:40	Animal fasted 18 hours; ready for removal of liver									
	8:40-8:55	Liver removed									
	9:10	Blood specimen taken; bladder emptied	51.7	10.63	0.32						
	10:10	Injected 0.5 gm. glucose and 1 cc. N/5 $NaHCO_3$ for each kgm. in 50 cc. physiologic salt solution; repeated injection									
	11:10	Repeated injection									
	p.m.										
	12:10	Blood specimen taken; urine catheterized; repeated injection	47.9	8.15	0.25	35	154	80	14	4.6	26.7
	1:10	Repeated injection									
	2:10	Repeated injection									
	3:10	Blood specimen taken; urine catheterized; repeated injection	44.1	6.43	0.90	70	301	185	25	8.3	61.7
	4:10	Repeated injection									
	5:10	Repeated injection									
	6:10	Blood specimen taken; urine catheterized	46.2	5.80	0.96	40	240	138	18	6.0	46.0

* The intravenous administration of $NaHCO_3$ appears to have no effect on the content of ammonia in the blood after hepatectomy, but it appears that the content of ammonia in the urine is considerably decreased. This table should be compared to table 4, in which a similar experiment is recorded except that HCl was injected.

hydrogen-ion concentration of the urine. Within a few hours after the animal recovers from the anesthetic there is a decrease of ammonia in the urine accompanied by an increase in the carbon dioxide combining power of the blood and hydrogen-ion concentration of the urine. These changes in the amount of ammonia are definite when the hourly excretion

in milligrams of ammonia nitrogen is considered. When the relative amount of ammonia is considered with reference to the total nitrogen or urea excreted, it is observed that there is a great increase in the relative amount of ammonia excreted, since following hepatectomy there is a progressive decrease of urea in the urine. The diminishing amount of urea in the urine does not seem to affect the absolute amount of ammonia excreted until the urea in the urine has diminished to about the same level as the ammonia. When this very low level of excretion of urea is reached the urea in the blood has also decreased to minimal values, and the amount of ammonia excreted in the urine is markedly reduced. This disappearance of ammonia and urea from the urine usually occurs from twenty-four to thirty-six hours after hepatectomy, although the interval after hepatectomy may be decreased by the extensive diuresis following intravenous injections of large amounts of diuretic fluids.

The ammonia in the blood is usually slightly increased after hepatectomy. The amount of ammonia in the blood, however, is variable in different animals and varies from time to time in the same animal, although the entire amount present is small. This variation does not appear to bear a direct relationship to the excretion of ammonia by the kidney since the amount of ammonia in the blood may be greater or less during periods of excretion of much or little ammonia. It should also be noted that at the time a minimal amount of urea and ammonia is excreted in the urine the urea in the blood is minimal, but the ammonia in the blood is usually considerably above normal. The ammonia in the tissues (muscular) appears to be comparable to that in the blood after hepatectomy, although the values obtained tend to be slightly higher in the tissues than in the blood.

If both kidneys are removed at the same time hepatectomy is performed, the ammonia in the blood increases, but the increase is of the same order of magnitude as that which is found in the blood of animals after hepatectomy alone. There is usually a roughly progressive increase of ammonia in the blood, but the increase is never more than 1.5 mgm. for each 100 cc. of blood eighteen hours after bilateral nephrectomy and hepatectomy. The ammonia in the muscles has been found to be only slightly higher than that in the blood. It would appear that fermentation in the gastro-intestinal tract may give rise to considerable ammonia. In a few animals in which the entire gastro-intestinal tract, except a few centimeters of the terminal section, was removed at the same time hepatectomy and nephrectomy were performed, less ammonia appeared in the blood than when the gastro-intestinal tract was not removed. For the most part, however, these experiments were not conclusive since considerable ammonia was usually found in the few centimeters of large bowel which was not removed.

The amount of ammonia in the urine following hepatectomy is dependent

on the acid-base equilibrium of the blood. The intravenous administration of alkalis (sodium bicarbonate) markedly reduces the amount of ammonia in the urine, but the ammonia in the blood is not affected. The intra-

TABLE 4

*Effect of intravenous injection of acid after hepatectomy (dog 3, weighing 14.8 kgm.)**

DATE	TIME	COMMENT	BLOOD			URINE					
			Carbon dioxide combining power	Urea nitrogen and ammonia nitrogen	Ammonia nitrogen	Volume	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Ammonia nitrogen each hour	Urea nitrogen each hour
	a.m.		*volumes per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
4-26-26	8:40	Animal fasted 18 hours; ready for removal of liver									
	8:40–8:55	Liver removed									
	9:15	Blood specimen taken; bladder emptied	53.6	9.75	0.12						
	9:35	Injected 0.5 gm. glucose and 2 cc. N/5 HCl for each kgm. in 50 cc. physiologic solution sodium chloride									
	10:30	Injection repeated									
	11:15	Injection repeated									
	p.m.										
	12:15	Blood specimen taken; urine catheterized; injection repeated	39.2	8.40	0.25	80	240	160	16	5.3	53.3
	1:15	Injection repeated									
	2:15	Injection repeated									
	3:15	Blood specimen taken; urine catheterized; injection repeated	36.5	2.60	0.21	65	305	95	35	11.7	31.7
	4:15	Injection repeated									
	5:15	Injection repeated									
	6:15	Blood specimen taken; urine catheterized; injection repeated	23.6	1.86	0.20	150	390	99	51	17.0	33.0
	7:15	Injection repeated									
	8:15	Injection repeated									
	9:15	Blood specimen taken; urine catheterized; injection repeated	22.8	1.83	0.24	150	345	84	27	9.0	28.0

* The intravenous injection of HCl seems to have no effect on the content of ammonia in the blood after hepatectomy, but the content of ammonia in the urine is increased. The experiments of tables 3 and 4 may be compared because the volume and the content of urea of the urine passed following hepatectomy are similar in both.

venous injection of acid (hydrochloric) causes more ammonia to be excreted without apparent changes in the amount in the blood. Neither injections of acid nor of alkali produce exactly quantitative changes in amount of

ammonia in the urine, but the changes in the hepatectomized animal are similar to those in the normal animal after similar injections.

The injection of ammonium salts (chloride or carbonate) into normal dogs is followed by rapid conversion of most of the ammonia into urea.

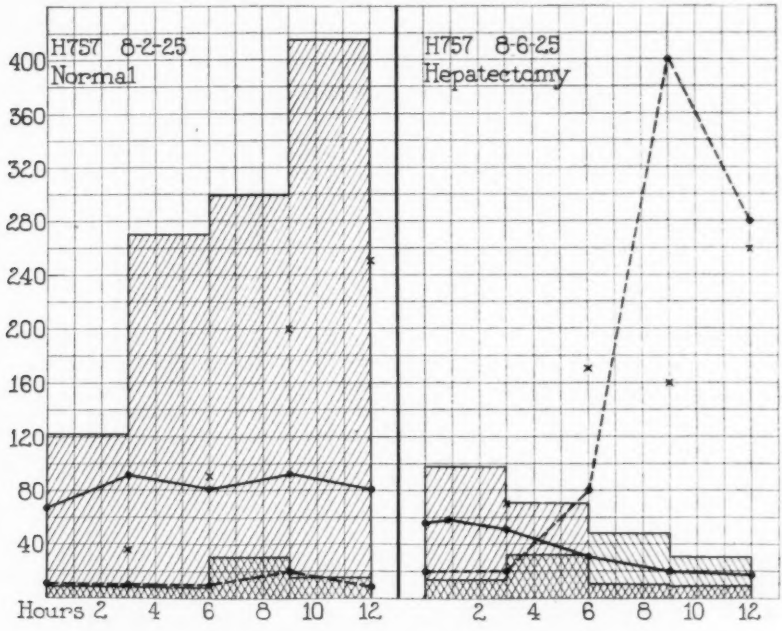

Fig. 1. Curves showing the formation of urea from ammonia in the normal animal and the absence of formation of urea under similar conditions after hepatectomy. The solid line represents the blood urea nitrogen and the broken line the blood ammonia nitrogen (x100) in milligrams for each liter of blood. The rectangles hatched from right to left represent the milligrams of urea nitrogen and those hatched from left to right represent the milligrams of ammonia nitrogen excreted each hour of the intervals when the urine was collected. The mark *X* represents the number of cubic centimeters of urine for the previous three-hour period. Intravenous injections of glucose were the same in each experiment and 416 mgm. of ammonia nitrogen as ammonium chloride was administered six hours after the mock operation in the first case and six hours after hepatectomy in the second. The marked decrease in urea and increase in ammonia in the blood and urine following hepatectomy may be noted (tables 1 and 2).

Less than 20 per cent of the injected ammonia has been recovered as ammonia in the urine, and the remainder has been converted into urea, the excess of which is found in the urine. The increased amount of ammonia in the blood after the injection is of short duration, since normal values are found

within an hour. We have used 26 mgm. of ammonia nitrogen for each kilogram of body weight as a standard dose. The injection of this amount into nephrectomized dogs is followed by a marked increase of urea in the blood and the disappearance of the excess ammonia from the blood within an hour. Following the injection of this material into hepatectomized

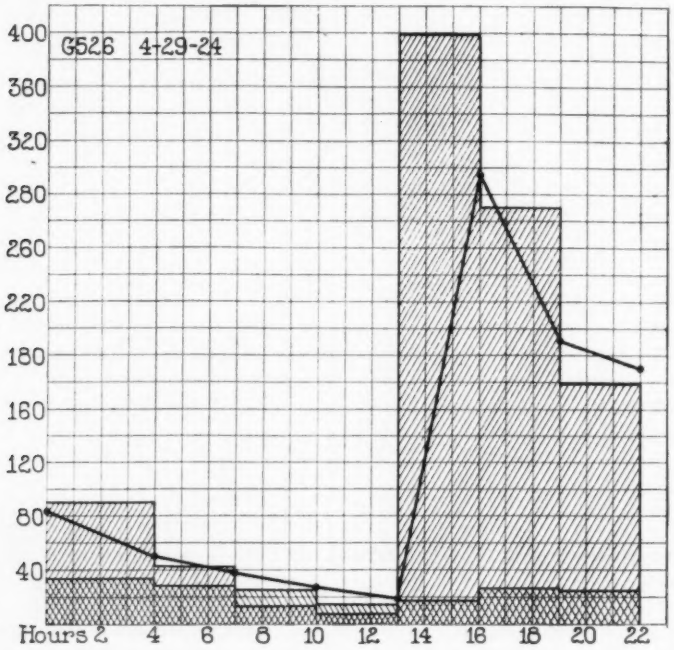

Fig. 2. Curves showing the formation of ammonia from urea in the hepatectomized animal. The solid line represents the blood urea nitrogen expressed in milligrams for each liter of blood. The rectangles hatched from right to left represent the milligrams of urea nitrogen and those hatched from left to right represent the milligrams of ammonia nitrogen excreted each hour of the intervals when the urine was collected. The marked decrease of urea in the blood and of urea and ammonia in the urine and the increase in each following the intravenous injection of 17.6 grams of urea thirteen hours after hepatectomy may be noted (tables 5 and 6).

dogs the ammonia in the urine is increased so that about 60 per cent of the amount injected is recovered in the urine within six hours after injection, and a change is not produced in the amount of urea in the urine. There is an increased amount of ammonia in the blood after injection, but only a slight decrease occurs several hours after injection, and the urea in the blood is not altered. If ammonia is injected into animals whose kidneys

and livers have been removed, the increase in ammonia in the blood and tissues is more persistent, but there is no increase in the amount of urea in the blood or tissues several hours after the ammonia is injected (fig. 1).

Injections of urea into dogs after hepatectomy do not produce any immediate effect on the ammonia in the blood or urine unless the injection is given at a time when the urea in the blood and urine has been reduced to an almost negligible amount. When a dog has excreted considerable urea

TABLE 5

*The changes in urea and ammonia nitrogen of the urine after hepatectomy (dog 4, weighing 13.6 kgm.)**

DATE	TIME	COMMENT	BLOOD—UREA NITROGEN	URINE				
				Volume	Urea nitrogen	Ammonia nitrogen	Ammonia nitrogen each hour	Urea nitrogen each hour
	a.m.		*mgm. per 100 cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
3-13-24	8:50	Animal fasted 18 hours; ready for removal of liver						
	8:50–9:00	Blood specimen taken; bladder emptied	9 0					
	9:05–9:30	Liver removed						
	11:50	Blood specimen taken; urine catheterized	7 8	34 0	215 0	18 6	6 2	71 7
	p.m.							
	2:50	Blood specimen taken; urine catheterized	5 3	60 0	204 0	55 2	18 4	68 0
	5:50	Blood specimen taken; urine catheterized	3 35	38 0	81 5	47 5	15 8	27 2
	8:50	Blood specimen taken; urine catheterized	2 15	36 0	50 1	12 5	4 2	16 7
	11:50	Blood specimen taken; urine catheterized	1 98	21 0	18 3	10 7	3 6	6 1
	a.m.							
3-14-24	2:50	Blood specimen taken; urine catheterized	2 67	17 0	9 35	8 5	2 8	3 1
	5:50	Blood specimen taken; urine catheterized	2 32	14 5	7 1	6 6	2 2	2 7
	8:50	Blood specimen taken; urine catheterized	1 68	18 0	5 46	7 5	2 5	1 8
	10:50	Blood specimen taken; urine catheterized	1 52	23 0	2 66	7 8	2 6	1 3

* Thirty cubic centimeters of 10 per cent glucose in physiologic solution sodium chloride were injected each hour after hepatectomy. It is to be noted that the urea content of the urine decreases when the preformed urea is washed from the blood and tissues as is evidenced by the decreased urea of the blood. The excretion of ammonia is also reduced when the content of urea in the urine is so extremely lowered. This experiment is to be compared to that recorded in table 6.

over a period of eighteen to thirty hours after the liver has been removed the urea in the blood and tissues is reduced below 1.5 to 2 mgm. of urea nitrogen for each 100 cc. of blood or 100 grams of tissue, the excretion of ammonia in the urine which has been constant before is markedly reduced, whereas the amount of ammonia in the blood is considerably above normal. The intravenous injection of urea at this time with the resultant increase of urea in the blood, tissues and urine gives rise to a marked increase of ammonia in the urine without any appreciable change in the amount of

ammonia in the blood or tissues. The increase of ammonia in the urine is usually to a point slightly above the level of the excretion of ammonia before the depletion of the urea in the blood and tissues (fig. 2).

The amino acids of the blood, urine and tissues increase after hepatectomy and the amino acids of the urine are above normal even after the depletion of the urea and decrease in the ammonia in the urine. The injection of amino acids at this time, or at any other time, does not alter the amount of urea or ammonia in the blood or urine; there is no evidence

TABLE 6

*Formation of ammonia from urea after hepatectomy (dog 5, weighing 19.3 kgm.)**

DATE	TIME	COMMENT	BLOOD—UREA NITROGEN	URINE				
				Volume	Urea nitrogen	Ammonia nitrogen	Ammonia nitrogen each hour	Urea nitrogen each hour
	a.m.		*mgm. per 100 cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
4-29-24	8:45	Animal fasted 18 hours; ready for removal of liver; blood specimen taken; bladder emptied	8.2					
	9:00-9:30	Liver removed						
	p.m.							
	12:15	Blood specimen taken; urine catheterized	4.9	456	329	123.0	33.7	89.7
	4:00	Blood specimen taken; urine catheterized	3.8	180	135	90.0	28.6	42.8
	6:50	Blood specimen taken; urine catheterized	2.7	352	37.0	75.0	26.7	12.9
	10:00	Blood specimen taken; urine catheterized; injected 17.6 gm. urea	2.0	382	25.3	49.7	15.7	8.0
	a.m.							
4-30-24	1:00	Blood specimen taken; urine catheterized	33.4	546	1317.0	54.6	18.2	439.1
	4:00	Blood specimen taken; urine catheterized	21.0	280	931.0	79.0	26.4	310.3
	7:00	Blood specimen taken; urine catheterized	19.0	265	538.0	76.8	25.6	179.4

* One hundred fifty cubic centimeters 10 per cent glucose, 1 per cent Na_2SO_4 were injected each hour after hepatectomy. With the decreasing amount of urea in the urine following hepatectomy and the coincident decrease in ammonia further decreases (as in table 5) would have occurred were it not for the injection of urea. Following injection of urea there was a definite increase in the urea and ammonia of the urine

that ammonia or urea can be formed from amino acids in the absence of the liver.

COMMENT. Besides the foregoing chemical evidence that urea does not form from ammonia in the dehepatized dog, we have had many examples of the increased susceptibility of these animals to intoxication following the administration of ammonia. Amounts of ammonia which do not produce untoward symptoms in normal dogs must be injected at a greatly reduced rate after hepatectomy. It would appear that the entire organism reacts severely to concentrations of ammonium salts of more than 2 mgm. of

ammonia nitrogen for each 100 cc. of blood. Large amounts of ammonium salts may be administered to normal dogs unless the rate of injection is rapid enough to allow this accumulation of ammonia in the blood before it can be converted to urea or be excreted. After hepatectomy ammonia must be injected considerably slower if the animal is to survive. Even with repeated small injections, symptoms appear when the amount of ammonia in the blood approaches 2 mgm. for each 100 cc.

Because urea is not synthesized by the dehepatized dog, this type of experiment is an excellent preparation for the study of the formation and excretion of ammonia. It would appear that the ammonia in the blood arises principally in the gastro-intestinal tract, either from fermentation of food and intestinal juices or from urea. In view of the rapid conversion of ammonia to urea in the normal animal it would appear that only small amounts of ammonia thus formed would escape conversion to urea as the portal blood passes through the liver. The lack of correlation between the amount of ammonia in the blood and tissues after hepatectomy and the amount of ammonia in the urine is also suggestive that the ammonia in the urine is not directly related to the ammonia in the blood. The alterations in the ammonia in the urine produced by the administration of fixed base or acid are similar in normal or dehepatized dogs, and it would appear that if sufficient urea is present the liver is not specifically involved in the formation of the ammonia concerned with the regulation of the acid-base balance of the body.

The decrease of ammonia in the urine concomitant with the depletion of urea in the urine after hepatectomy is particularly significant. At this time amino acids, uric acid and creatinine are being formed in the tissues at approximately normal rates and protein catabolism is continuing except for the entire failure of conversion of the liberated (or injected) amino acids to urea. The decrease in ammonia does not occur until the urea has almost been eliminated from the urine, but the other nitrogenous metabolites are not decreased and it would appear that ammonia in the urine is not formed directly from amino acids, uric acid or creatinine. The reappearance of ammonia with the increase in urea appears as direct proof of the origin of ammonia in the urine from urea. Although these observations do not entirely preclude the possibility of some other precursors of ammonia in the urine, at least it may be said that any such precursor is directly dependent on the urea in the blood and urine.

SUMMARY

After removal of the dog's liver there is complete cessation of the formation of urea and the administration of ammonia is followed by its recovery in the urine and tissues as ammonium salts without conversion to urea, as is found in normal animals. Because of this failure of formation of urea

after removal of the liver, the ammonia in the blood and tissues is usually found to be considerably increased. A considerable portion of this ammonia apparently arises from the gastro-intestinal tract and appears to be quite independent of the ammonia in the urine. The ammonia in the urine following hepatectomy is dependent on the acid-base equilibrium of the body to about the same extent as is found in normal animals. The liver does not appear to be necessary for the regulation of neutrality which is concerned in the formation of ammonia in the urine if sufficient urea is present.

The urea in the blood, tissues and urine progressively decreases following hepatectomy and as the amount of preformed urea in the body becomes depleted the concentration of urea in the urine becomes extremely low. With but minimal amounts of urea in the urine the ammonia in the urine is likewise decreased, although large amounts of amino acids, uric acid and creatinine are present. The administration of these substances is without effect on the amount of ammonia in the urine, but the administration of urea is followed by a marked increase of the ammonia in the urine. This appears to be proof that urea is the origin of ammonia in the urine (tables 1 to 6).

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THE RÔLE OF HEXOSE DIPHOSPHATE IN MUSCLE ACTIVITY

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The purpose of this work is to show that hexose diphosphate increases muscular efficiency and that this increase may be due to its function as a muscle buffer rather than to its accredited function as "lactacidogen," the immediate precursor of lactic acid in the muscular contraction.

Hexose diphosphate was discovered by Iwanoff in 1906 (1), and isolated by Young in 1909 (2), as an intermediary product involved in the accelerating action of phosphate on the yeast fermentation of sugar. Embden in 1912 (3), found that lactic acid in muscle extract increased on incubation. He suggested the name "lactacidogen" for the as yet undiscovered substance from which the lactic acid was derived. In 1914 (4), he found that the inorganic phosphate of muscle extract also increased on incubation, corroborating the work of Weyl and Zeitler in 1882 (5). Further investigations revealed the fact that hexose diphosphate yielded increases in both lactic acid and inorganic phosphate when added to muscle extract, while other compounds which he treated did not . Accordingly he concluded that hexose diphosphate was "das Lactacidogen," the precursor of lactic acid in the muscular contraction, (4). In 1921 (6), he found that feeding glucose and acid sodium phosphate increased the efficiency of both animals and humans. These results were not corroborated by Flynn (7), who concluded that any changes observed could be accounted for by the tonic effect of phosphate on the alimentary tract.

The question of the effect of hexose diphosphate on muscular efficiency is still in doubt since it rests upon ingestion experiments in which the side actions of phosphate upon the alimentary tract can not be excluded. The question of its function as lactacidogen rests upon the observation that adding hexose diphosphate to muscle extract increases the yield of lactic acid and inorganic phosphate.

In the experiments to be reported in this paper the effect of hexose diphosphate on muscular efficiency was studied by perfusing an intact, isolated muscle the efficiency of which could be measured. The mechanism of the action of hexose diphosphate was analyzed by perfusing the muscle with its components, glucose + phosphate, glucose, and phosphate solutions.

The frog's heart was chosen for perfusion chiefly because of its availability

[1] This article represents a part of the work done in fulfillment of the thesis requirement for the degree of Doctor of Medicine at Yale University School of Medicine.

and the well-known standard techniques of perfusion, as well as the fact that the influence of electrolytes, pH, oxygen, load and temperature on the frog's heart have been extensively studied. With due consideration of these factors, a solution of hexose diphosphate was perfused through the beating frog's heart, solutions of glucose, phosphate, and glucose + phosphate being used as controls. The muscular efficiency was measured directly in terms of amplitude of contraction by means of a heart lever writing on a smoked drum.

Preparation of solutions. The calcium salt of hexose diphosphate, $(C_6H_{10}O_4)Ca(PO_4)_2 \cdot 2H_2O$, is produced by Bayer & Co., of Germany under the trade name "Candiolin." A considerable quantity of this salt was obtained through the kindness of the Winthrop Chemical Co., of New York City. The commercial product was purified by the method of Neuberg (8), being tested for purity by the method he describes. Since the calcium salt is only sparingly soluble in water, the sodium salt was made by Neuberg's method. Great care was taken to secure an exact precipitation of the calcium by sodium oxalate without an excess of oxalate, as Clark (9) has shown that minute traces of calcium have a decided influence on the frog's heart, while excess oxalate will precipitate the calcium from the Ringer's perfusion fluid. The final clear solution was tested quantitatively for organic phosphorus by the Fiske and Subbarow method (11), and diluted to contain 69 mgm. of phosphorus per 100 cc. This solution contains the equivalent of 200 mgm. of glucose per 100 cc.

Throughout the experiments Clark's modification of Ringer's solution (NaCl 0.65 per cent, KCl 0.014 per cent, $CaCl_2$ 0.012 per cent, $NaHCO_3$ 0.01 per cent, and NaH_2PO_4 0.001 per cent) was used with one alteration; the acid sodium phosphate was omitted. Highest purity chemicals were used throughout. The solutions were made up to double strength in large quantities and diluted either with an equal volume of water or with the solution to be studied, which had also been made up to double strength. In making up the Ringer's solution to double strength, precipitation of the $CaCO_3$ was avoided by stirring constantly during the addition of the $CaCl_2$ to the NaCl, KCl, and $NaHCO_3$, which had been dissolved in about 75 per cent of the required amount of distilled water. Five drops of Cresol Red indicator solution were added to each 100 cc. of this double strength Ringer's solution.

The glucose solution was made to contain 200 mgm. per 100 cc., the equivalent of the glucose in the hexose diphosphate solution.

The phosphate solutions were made up, with sodium phosphate and sodium biphosphate in proportions which would necessitate minimal additions of acid or alkali to bring them to the desired pH, to contain 69 mgm. of phosphorus per 100 cc., the equivalent of the phosphorus in the hexose diphosphate solution.

The glucose + phosphate solution was made up by dissolving 200 mgm. of glucose in 100 cc. of the phosphate solution.

It will be seen that in the final dilutions the concentrations of all the electrolytes were equal and the concentration of glucose equivalent to that of the hexose diphosphate solution.

The hydrogen ion concentration in the perfusion system was regulated by means of 0.1 N NaOH and 0.1 N HCl with cresol red as an indicator. Cresol red four times as concentrated as that used in these experiments seemed to have no deleterious influence on the frog's heart. Five drops of this indicator were added to each 1000 cc. of double strength solution and one drop to each 100 cc. of the final dilution. The optimum pH for the frog's heart is well on the alkaline side of neutrality, but at a

pH of 7.8 calcium phosphate would be precipitated from the solutions used. Accordingly the pH was regulated to about 7.5 at which the indicator shows a definite pink on a yellow background. All the solutions used in a single experiment were brought to the same pH just before perfusion.

The solutions were perfused at room temperature. No effort was made to oxygenate them. However, they were made up simultaneously and at the same temperature, so that the amount of oxygen dissolved in the solutions would be approximately the same throughout.

Technique of perfusion. Medium sized female frogs weighing between 40 and 60 grams, which had been starved approximately 2 months before the experiments, were used.

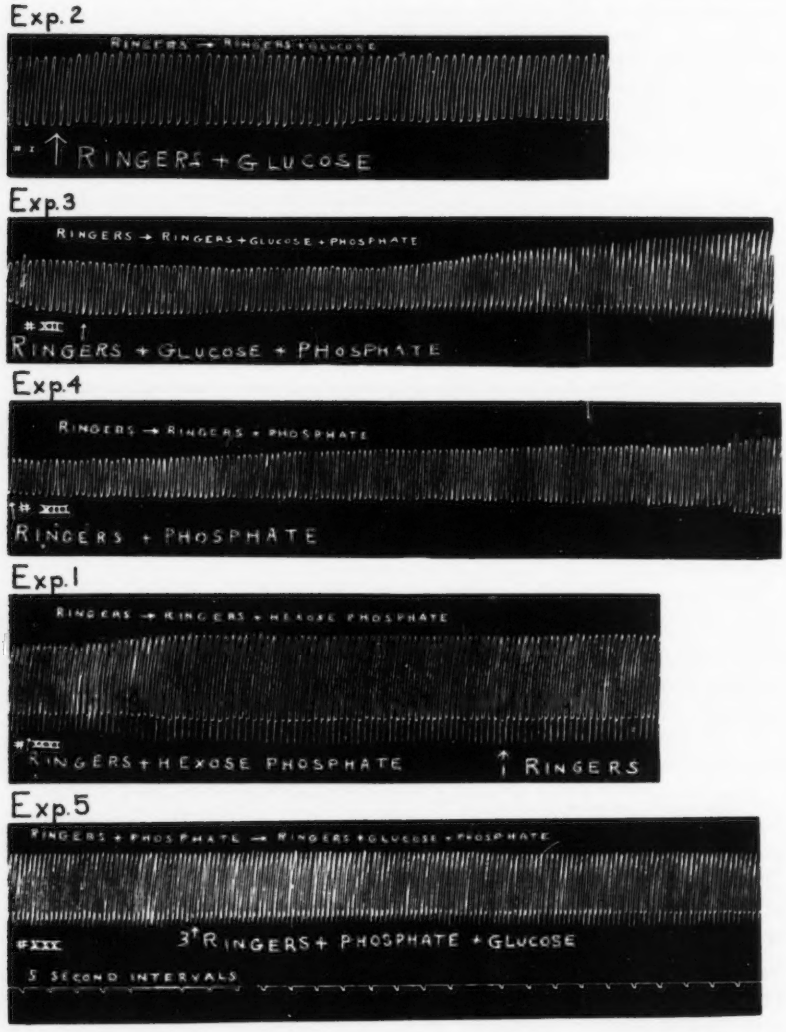
The frog was pithed, both brain and cord, the shoulder girdle severed and the pericardial sac opened. The right superior vena cava, right aorta and left superior vena cava were ligated and small glass cannulae tied into the left aorta and inferior vena cava, care being taken to prevent air from getting into the system. The cannula in the inferior vena cava was then attached to the outflow rubber tubing of a Simpson constant pressure perfusing apparatus (12), and Ringer's solution was perfused through the system, the "venous" pressure being controlled by a thumbscrew clamp. The rate of flow was roughly measured in drops per beat from the aortic cannula. When the perfusion was run to obtain a graphic record a rate of one beat per drop was maintained. In any one series the rate was unchanged. The heart was fastened by a silk ligature passed through the apex of the ventricle to a "Harvard" heart lever which recorded the heart's action on a smoked kymograph drum so that the upstroke represented a systole. From these graphs, some of which are shown in the accompanying charts, measurements were made of the rate and amplitude of the heart contractions. The product of the rate in beats per minute and the amplitude in millimeters was taken as a relative index of cardiac efficiency.

EXPERIMENTAL RESULTS. The graphs in the figure and the measurements in the table are typical examples of the results encountered. These results are only relative, those found in one experiment not being quantitatively comparable to those found in others as different frogs were used for each. The significant findings are limited to the effect observable in any one experiment on adding one new component to the perfusion fluid. The degree of change is only roughly significant.

Experiment 1. Effect of adding hexose diphosphate to Ringer's perfusion fluid. After the heart had been perfused for an hour with Ringer's solution at one beat per drop, Ringer's + hexose diphosphate was introduced. Graph 1 shows the transition point. An immediate increase in amplitude of contraction is evident. In this case the rate decreases, probably because the hexose diphosphate solution had been in the ice-box before it was used for the perfusion. Accordingly the measurements taken from another graph in which there is an increase in rate as well as in amplitude are also given in the table as experiment 1a.

Experiment 2. Effect of adding glucose to Ringer's perfusion fluid. The heart was perfused for an hour with Ringer's solution at one beat per drop before changing to Ringer's + glucose. No evidence of any significant change in rate or amplitude is evident. (See graph 2 and table.)

Experiment 3. Effect of adding phosphate to Ringer's + glucose perfusion fluid. The heart was perfused for an hour with Ringer's + glucose at one



beat per drop before changing to Ringer's + glucose + phosphate. Although no significant effect was observed on changing from Ringer's to Ringer's + glucose as shown in the preceding graph, on adding phosphate

to the solution, a very considerable increase in the amplitude of contraction was observed. This increase is much greater than that observed on adding hexose diphosphate as shown in the first graph. As pointed out before, different frogs were used in the two experiments and only the qualitative change on supplying a new component is of significance. See graph 3 and experiment 3 in table.

4. Effect of adding phosphate to Ringer's perfusion fluid. The heart was perfused with Ringer's solution at one beat per drop before changing to Ringer's + phosphate. Upon adding the phosphate the amplitude was decidedly increased just as in the preceding experiment when glucose + phosphate was added. See graph 4 and experiment 4 in table.

5. Effect of adding glucose to Ringer's + phosphate perfusion fluid. The heart was perfused for an hour at one beat per drop with Ringer's + phosphate before glucose was added to the solution. No noticeable effect is observed on adding the glucose. See graph 5 and experiment 5 in table.

Discussion. In these experiments a visible increase of amplitude together with an increase in the product of the rate in beats per minute multiplied by the amplitude in millimeters, was considered an indication of increased muscular efficiency. Hexose diphosphate does increase muscular efficiency. A molecular equivalent solution of glucose + phosphate also increases efficiency. On the basis of the lactacidogen theory it was believed that hexose diphosphate might be formed in the muscle from glucose and phosphate supplied to it. This belief was substantiated in that no demonstrable increase in efficiency was noted on adding glucose to the perfusion fluid. However, upon adding phosphate to the perfusing solution, an increase in efficiency was easily demonstrable. The lactacidogen theory was still tenable on the supposition that sufficient glucose was present in the heart to unite with the phosphate leading to the formation of lactacidogen. An attempt was accordingly made to exhaust the heart's store of glucose by perfusing with a phosphate solution and then to determine whether a glucose + phosphate solution noticeably increased efficiency. No increase was observed. Therefore efficiency was always increased when phosphate was added and never when phosphate in some form was not added.

In connection with another part of the work, analyses were made of the perfusion fluids. It was found that before perfusion no inorganic phosphate could be detected in the hexose-diphosphate solution by the Bell-Doisy method (13). After perfusion, at a slow rate of 6 beats per drop, a considerable trace of inorganic phosphate was found. Evidently the presence of inorganic phosphate was associated with the increases in muscular efficiency.

It will be remembered that cresol red was added as an indicator to all the perfusing solutions. At pH 7.5, the color resulting would be a definite pink on a yellow background. It was noticed that the perfusate of Ringer's

solution or of Ringer's + glucose which was being collected for analysis, after perfusing at the slow rate of 6 beats per drop, was definitely yellow, or more acid. On the other hand, when phosphate in any form was added to the Ringer's solution, the fluid after perfusion was still a faint pink on a yellow background, more acid than before perfusion, but considerably less acid than the Ringer's solution after perfusion. There was a positive correlation of increase of muscular efficiency, presence of phosphate in the perfusate and lessened change of pH. Hill (15) has pointed out the necessity of oxygen for the removal of lactic acid. Since these solutions were not oxygenated, it is possible to think of considerable amounts of lactic acid accumulating, rendering the pH of the perfusate more acid and diminishing

TABLE 1

EXPERIMENT	PERFUSION SOLUTION	CONTRACTION		
		Rate beat per minute	Amplitude	Rate × Amplitude
			mm.	
1	Ringer	68	18.0	1,224
	Ringer + hexose diphosphate	62	21.0	1,330
1a	Ringer	33	17.2	567
	Ringer + hexose diphosphate	42	19.2	806
2	Ringer	37	18.2	672
	Ringer + glucose	40	17.1	695
3	Ringer + glucose	46	15.3	702
	Ringer + glucose + phosphate	57	13.2	1,336
4	Ringer	56	13.6	762
	Ringer + phosphate	56	22.2	1,245
5	Ringer + phosphate	65	16.8	1,094
	Ringer + phosphate + glucose	65	15.8	1,030

muscular efficiency. With the presence of phosphate in the solution, the acidity would be lessened through the buffer action of the phosphate. With the lessening of the acidity there would be an increase in the muscular efficiency.

This explanation holds for the increases in efficiency noted with the inorganic phosphate solutions. Hexose diphosphate can be split into inorganic phosphate and lactic acid as shown by Embden (4), and here it is shown that a living intact muscle can split off inorganic phosphate. Hexose diphosphate seems to be a "biological" compound which is capable of being split by muscle tissue. The question naturally comes up whether hexose diphosphate may not itself act as a buffer besides being able to yield inorganic phosphate for buffering.

Using Meyerhof's figures for the dissociation constants of hexose diphosphoric acid (16), the efficacy of hexose diphosphate as a buffer was calculated in the following manner:

Henderson's (17) equation for the concentration of hydrogen ions in a buffer salt solution formed by the reaction of a strong base and weak acid is

$$(1)\ \mathrm{pH} = \mathrm{pK'} - \log \frac{\text{concentration of salt}}{\text{concentration of acid}} \quad \text{or} \quad \mathrm{cH} = \mathrm{K'} \times \frac{\text{concentration of acid}}{\text{concentration of salt}}$$

The second activity dissociation constant of hexose diphosphoric acid, taken from Meyerhof's figures (16) is

$$(2) \qquad \mathrm{pK'_2} = 6.32 \text{ or } \mathrm{K'_2} = 4.786 \times 10^{-7}$$

The second activity dissociation constant of orthophosphoric acid, taken from Meyerhof's figures (16) is

$$(3) \qquad \mathrm{pK'_2} = 6.8 \text{ or } \mathrm{K'_2} = 1.58 \times 10^{-7}$$

The dissociation constant of lactic acid, taken from Clark's figures (19) is

$$(4) \qquad \mathrm{K'_1} = 1.4 \times 10^{-4}$$

The pH of resting muscle, taken from Meyerhof's figures (180) is

$$(5) \qquad \mathrm{pH} = 7.2 \text{ or } \mathrm{cH} = 6.31 \times 10^{-8}$$

Starting from pH 7.2, Meyerhof's figure for the pH of resting muscle, the proportions of acid to basic sodium hexose diphosphate were calculated using Henderson's equation given above, and Meyerhof's figure for the second dissociation constants of the two phosphoric acids.

$$(6) \qquad \mathrm{cH} = \mathrm{K'_2} \times \frac{\mathrm{C_6H_{10}O_4\ (NaH\ PO_4)_2}}{\mathrm{C_6H_{10}O_4\ \ (Na_2PO_4)_2}}$$

Substituting the values given above in (2) and (5) in this equation,—

$$(7) \qquad 6.31 \times 10^{-8} = 4.786 \times 10^{-7}\ \frac{(1 - x)}{x}$$

and solving for x,—

$$(8) \qquad x = 0.883 \quad 1 - x = 0.117$$

Changing the value of x to 1 we have the proportion,

$$(9) \qquad \frac{\mathrm{C_6H_{10}O_4\ (NaH\ PO)_2}}{\mathrm{C_6H_{10}O_4\ (Na_2PO_4)_4}} = \frac{0.132}{1.0}$$

Assuming complete change of this mixture of hexose diphosphates to inorganic phosphate and lactic acid:—

$$0.132\ C_6H_{10}O_4\ (NaHPO_4)_2 + 1.0\ C_6H_{12}O_4\ (Na_2PO_4)_2 =$$
$$(10)\quad x\ Na\ C_3H_5O_3 + (2.264 - x)\ C_3H_6O_3 + y\ Na_2H\ PO_4 + (2.264 - y)NaH_2PO_4$$

since, on complete hydrolysis of the amounts of hexose diphosphates above, 2.264 moles of lactic acid and 2.264 moles of inorganic phosphate are formed.

There is finally an equilibrium reached for the distribution of sodium at a fixed pH, so that by using Henderson's equation again, we can calculate the theoretical proportions of lactic acid to sodium lactate and sodium mono- to di-basic phosphate.

$$(11)\qquad cH = 1.4 \times 10^{-4}\ \frac{(2.264 - x)}{x} = 1.58 \times 10^{-7}\ \frac{(2.264 - y)}{y}$$

The amount of sodium present in equation (10) above is 4.264 mols. Since x, the amount of sodium lactate, has one sodium equivalent attached to it, (2.264-y) the amount of sodium acid phosphate, one equivalent; and y, the amount of sodium basic phosphate has 2 equivalents attached to it, we have the simultaneous equation for sodium.

$$(12)\qquad x + (2.264 - y) + 2y = 4.264 \text{ or } x + y = 2$$

Solving for x and y in equations (11) and (12) we have the proportions of lactic acid to sodium lactate and sodium acid to basic phosphate.

$$(13)\qquad \frac{C_3H_6O_3}{NaC_3H_5O_3} = \frac{0.282}{1.9821} \text{ and } \frac{NaH_2PO_4}{Na_2H\ PO_4} = \frac{2.246}{0.01789}$$

Using Henderson's equation to calculate the cH from these values, from the proportion of lactic acid to sodium lactate:—

$$(14)\qquad cH = 1.4 \times 10^{-4}\ \frac{0.282}{1.9821} = 1.99 \times 10^{-5}\ pH = 4.7$$

Therefore, starting from a cH of 6.31×10^{-8} or pH 7.2, if a mixture of hexose diphosphate is completely hydrolyzed to lactic acid and inorganic phosphate, an equilibrium is reached at cH 1.99×10^{-5} or pH 4.7.

By a similar process the buffer value of undissociated hexose diphosphate against an equivalent amount of lactic acid can be estimated.

The calculated pH attained is found to equal 4.65 or cH to equal 2.23×10^{-5}.

Using amounts of phosphate and glucose equivalent to those present in the hexose diphosphate above, the relative buffer value of inorganic phosphate is determined, starting from the same pH. The pH is found to go to 4.25 or cH to 5.66×10^{-5}.

These calculations are for a homogeneous system containing only glucose and phosphate or their equivalents as hexose diphosphates or lactic acid. Identical amounts of lactic acid and phosphate have been used throughout. These equations show the deviation of cH as a result of the action of identical amounts of lactic acid on 1, completely hydrolyzed hexose diphosphate mixture; 2, unhydrolyzed hexose diphosphate mixture, and 3, an equivalent amount of phosphate mixture, starting from the same pH, 7.2.

I. With completely hydrolyzed hexose diphosphate mixture
cH 6.31×10^{-8} goes to cH 1.99×10^{-5}
pH 7.2 goes to pH 4.70

II. With unhydrolyzed hexose diphosphate mixture
cH 6.31×10^{-8} goes to cH 2.24×10^{-5}
pH 7.2 goes to pH 4.65

III. With an equivalent amount of phosphate mixture
cH 6.31×10^{-8} goes to cH 5.66×10^{-5}
pH 7.2 goes to pH 4.25

In other words, the cH attained on the hydrolysis of a glucose phosphate mixture at pH 7.2 is 2.84 times that attained on the hydrolysis of an equivalent amount of hexose diphosphate mixture, starting from the same pH, and 2.53 times that attained by adding the equivalent amount of lactic acid to the unhydrolyzed hexose diphosphate mixture.

Conversely, the resistance to change of pH upon the hydrolysis of an amount of glucose equivalent to that contained in a hexose diphosphate mixture at pH 7.2, is 2.84 times as great if hexose diphosphate, which may be split into glucose and phosphate, is present, as it is if an equimolecular quantity of glucose and phosphate is present at the same pH.

Therefore, under conditions presumably similar to those in living muscle, hexose diphosphate would more efficiently buffer lactic acid formed by its decomposition than would an equivalent amount of undecomposed phosphoric ester or of orthophosphate.

CONCLUSIONS

1. Hexose diphosphate increases muscular efficiency.
2. The mechanism may be due to its buffering action rather than to the fact that it is the immediate precursor of lactic acid in the muscle contraction.

The writer wishes to express his sincere appreciation to Dr. John P. Peters, John Slade Ely Professor of Medicine, for his untiring interest, thoughtful criticisms and valuable suggestions, and to Dr. David I. Hitchcock for his criticisms of the mathematical expressions employed in this work.

Since this article has been prepared for publication the article of Fiske and Subbarow (20) has appeared. They have isolated about 70 per cent of the labile phosphorus in muscle as phosphocreatine, and believe that almost all the labile phosphorus in the muscle is in this form. They have determined the second activity dissociation constant of phosphocreatine as 2.5×10^{-5}. To quote: "Consequently, the hydrolysis of phosphocreatine during muscular contraction would be accompanied by the liberation of a relatively large amount of base." Their conclusions as to the function of phosphocreatine are quite similar to those in this article for the function of hexose diphosphate, as a mechanism for neutralizing acid.

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ON THE TRUE ANOXIDATIVE AND OXIDATIVE HEAT PRODUCTION OF SMOOTH MUSCLE[1,2]

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Two summers ago a visit with Professor Beijerinck at his charming country home in Holland had, among other pleasant results, the effect of sending me back to the papers of that investigator (1) in which it was shown that of all reactants none is so sensitive for the testing of minute traces of oxygen as are certain species of photobacteria. And as I reflected upon that recent measurement of my countrymen, Harvey and Morrison (2), whereby it was shown that these luminescent organisms only cease to glow when the concentration of oxygen in their environment has been reduced below 0.0007 of 1 per cent (by volume at atmospheric pressure), it occurred to me that there probably are other physiological processes that likewise cease to function only when the oxygen concentration is equally low. If so, then many processes, that until now have been called anoxidative, may prove to be oxidative upon reinvestigation; or at least more oxidative than is now thought to be the case. Especially did I begin to feel this about my own experiments on the anaerobic heat production of muscle (4), (5).

Accordingly I cast about for a method whereby a medium otherwise normal for muscle could not only be made quite free of oxygen, but also to include a continuous test for the degree of this freedom at least to the point where photobacteria cease to glow. The method devised by Kautzky and Thiele (3) is such a method and it is interesting to note that these authors use the same reducing agent, sodiumhydrosulphite, as Beijerinck did in his experiments, and further that they make use of photobacteria as test-objects as Beijerinck recommended forty years ago. Without dwelling upon the merits of the Kautzky and Thiele method I wish to emphasize at this point that however completely free from oxygen an

[1] The experimental basis of this paper has been carried out by the author in collaboration with Mr. Frederick W. Light, Jr. and Mr. Nolan L. Kaltreider. More detailed accounts of the experiments will appear shortly, with their names as co-authors. Mr. Light has been recent, and Mr. Kaltreider present, recipient of a grant from The Henry Strong Denison Medical Foundation.

[2] Reported at the XIIIth International Physiological Congress held at Boston, U. S. A., August 22, 1929.

inert gas may be as it is delivered from the reducing chamber, the freedom of the muscle chamber from oxygen will depend 1, upon the amount of leakage in the gas-chain and in the muscle-chamber itself, and 2, upon the previous $[O_2]$ prevailing in the spaces included therein.

The presence of small amounts of oxygen in the experimental chamber usually has been recognized, and to prevent a possible utilization of this oxygen by the living cells, cyanide has been introduced with the belief that this reagent in so-called reversible doses inhibited tissue oxidation completely. But the difficulty with cyanide is to find for each preparation a completely effective and yet reversible dose and still have an uninjured preparation with which to proceed with the experiment. One is forever held up on one or the other of the two horns of a dilemma; the dose is reversible, but possibly not quite effective; or it is effective but has left the preparation injured.

For this reason I have abandoned the use of cyanide altogether and have introduced living photobacteria on thin gelatin- or peptone-agar plates into the gas chain at points so that, at will, the gas may be tested either before entering or directly upon leaving the muscle chamber. The time required to read the end-point of this test is the time required for dark adaptation of the observer's eye. This time, since the room (in which the work is done) is already dimly lighted, requires at the longest not more than five minutes. The test shows whether the gas entering, or leaving, the muscle chamber contains more, or less, than 0.001 per cent of oxygen. If the bacteria are seen at once to glow brightly the $[O_2]$ may be anything higher than a few thousandths of one per cent; if dark adaptation is required then the $[O_2]$ is nearly 0.001 per cent; if the glowing cannot be seen even dimly after 3 to 5 minutes' dark adaptation then the $[O_2]$ is certainly less than 0.001 per cent per volume at 1 atmosphere pressure. A volumetric analysis of a sample of gas in which the photobacteria did not glow was kindly made by Doctor Grollmann of our laboratory and the absence of oxygen up to the limit of the certainty of the apparatus, namely, 0.001 per cent, was confirmed.

The muscles used in the present experiments were the urinary bladder of the turtle isolated with its nerves, as already described by Light (5), and the turtle's pyloric sphincter isolated with its nerve plexus intact. Contractions were evoked in the bladder by weak induced tetanic currents applied to the nerves; in the pyloric ring contractions were evoked by a preliminary treatment with pilocarpine, whereby no electrical current at all was required. The preparations were kept at room temperatures throughout. The methods and procedures otherwise followed were similar to those used heretofore and need no further description (4), (5).

Mr. F. W. Light, Jr. collaborated in all the experiments with the urinary bladder-nerve preparations, Mr. N. L. Kaltreider in all those with the

gastric pyloric sphincter. Prof. S. R. Damon of the Laboratory of Bacteriology (Prof. W. W. Ford, director) of the School of Hygiene and Public Health, Baltimore, kindly kept our cultures of photobacteria going during most of the period of the investigation. Specimens of pure cultures of the bacteria were furnished us through the kind offices of Professors M. W. Beijerinck and A. J. Kluyvers (of the Laboratory of Microbiology of the Technical High School of Delft, Holland) and Prof. E. Newton Harvey and his associate, Dr. S. E. Hill, of Princeton, to all of whom I take this opportunity of expressing my warmest thanks.

Results. In table 1 only observations of muscles in oxygen-plenty are shown, and these are segregated into three groups. For, upon beginning observations upon a fresh muscle in oxygen-plenty, and before it has suffered any known avoidable manipulation that could reduce its normal activities, any one of several levels of heat production may be observed. The heat production may be relatively high, or low, or of moderate amount. This has puzzled me greatly. But finally a satisfactory explanation has been reached by supposing that the muscles at the start may be in any one of three conditions: *a*. The muscles may be in what I shall call *balanced breathing*, no fatigue products are present except those formed by the resting basal metabolism plus those of the contractions under way, and no more or less oxygen is consumed than is necessary to remove the products of that contraction. *b*. The muscle for some reason may have just lost, or suffered temporarily a diminution in its power to utilize oxygen, or it may be in a state of "over-ventilation." In either case its heat production, although in O_2-plenty, will be relatively low. (See first section table 1 for two cases of remarkably low h.p.) *c*. The muscle at the start for some reason, and for some time, has not been oxidizing its metabolites, with the result that the fibers are overloaded with oxidizable materials but, their oxidizing powers now being fully recovered, they are producing extraordinary quantities of heat. These muscles may be said to be suffering from *respiratory arrears*. This last condition is always the case with those that have been subjected to prolonged anaerobiosis, and just when they are again furnished with O_2-plenty. Their heat productions are far greater than any observed. As shown in table 1, both initial and delayed heat productions show these three grades of activity, although all the muscles are basking in a plentitude of oxygen.

In table 2 are summarized the observations on muscles while they were being subjected to oxygen-want. These fall into two main groups; those suffering from partial oxygen withdrawal and those suffering from the extremity of O_2-want. Those in partial O_2-want are arranged in two subdivisions, the first of which consist of the older observations already published (5) of bladder muscles in $[O_2]$ of say 0.1 per cent to 0.01 per cent, but which had been treated with "reversible" doses of cyanide in the belief

that the cyanide would abolish completely all oxidations. The second subdivision of the muscles in partial oxygen want represents those muscles that were subjected to oxygen withdrawal to a concentration less than 0.01 per cent but still definitely more than 0.001 per cent, but which had been given no cyanide treatment. The second main group in the table contains the readings for those muscles contracting in an atmosphere containing what is known to be less than 0.001 per cent, for either the

TABLE 1

Smooth muscles in oxygen-plenty

H. P. in gm.-cal. $\times 10^5$, per gm. tension

CONDITION OF MUSCLE	NUMBER OF (V)	TENSION (T)	HEAT PRODUCED	
			"Initial"	"Delayed"
		grams		
1. Fresh preparations, before O_2-withdrawal				
(a) Balanced breathing, or over-ventilated?	5	33	1.50	2.27
	14	35	1.97	1.93
	6	22	0.61	1.27
	5	48	1.76	2.86
	7	29	0.67	1.19
	7	49	1.96	1.58
	5	57	2.03	1.72
(b) Balanced breathing, or respiratory arrears?	2	38	3.25	3.89
	7	45	2.63	3.54
	11	19	2.67	4.37
	6	36	3.31	2.61
2. Recovering from O_2-want, early phases	28	37	3.3	4.3
	6	21	2.4	5.2
	3	3	4.1	7.8
	2	6	7.3	13.6
	1	6	2.1	8.5

test-photobacteria were not seen to glow, or (in one case) to glow only quite dimly after three minutes' dark adaptation.

There can be no doubt as to the meaning of the results in this table. 1. Cyanide in "reversible" doses does not stop oxidations if oxygen is present in concentrations above 0.01 of one per cent. 2. Oxidations do not necessarily stop even when the concentration is less than 0.01 per cent and no cyanide is used. 3. The heat productions are gradually reduced in both initial and delayed periods and finally approach a steady state, following O_2-removal, only when the $[O_2]$ has become distinctly less than 0.001 of one per cent by volume.

From these experiments one must conclude further:

1. Smooth muscle of turtle's urinary bladder and pyloric sphincter, when contracting in oxygen-plenty and in response to autonomic or to nervous stimulation, usually produces quantities of heat that are equivalent to those observed for other muscle organs of both smooth and cross-striped varieties.

2. When in an atmosphere of purified nitrogen containing oxygen, if any, in quantity less than 0.001 per cent by volume, these smooth muscles

TABLE 2

Heat response of smooth muscle in O_2-want

H. P. in gm.-cal. $\times 10^5$, per 1 gm. tension (T)

CONDITION OF MUSCLE	NUMBER OF (V)	T	HEAT PRODUCED	
			"Initial"	"Delayed"
		grams		
1. In partial O_2-want				
(a) Cyanide, > 0.01 per cent O_2	8	67	2.53	1.33
	5	46	1.69	0.41
	9	33	1.58	1.02
	6	13	3.40	2.08
Mean, when	S = 28		2.26	1.22
(b) $[O_2] < 0.01\%, > 0.001\%$ 60 secs. D.A., bact. glow	5	44	1.34	1.04
	6	11	2.51	2.72
2. In extreme O_2-want				
$[O_2] < 0.001$ per cent	6	10	0.32	0.42
3 Min. D.A. bact. glow	4	9	0.67	1.06
5 Min. D.A., no glow	8	13	0.30	0.70
$[O_2] < 0.001$ per cent,	5	47	0.35	0.45
$[O_2] < 0.001$ per cent (?)	6	68	0.68	0.81
Mean, when	S = 29	29	0.44	0.68

develop somewhat less heat during the phase of rising tension than they do in the period immediately following, and the heats of both "initial" and "delayed" phases are only a small fraction of what they are when the muscles are contracting in oxygen-plenty,—the initial heat being about $\frac{1}{4}$; the delayed heat being about $\frac{1}{3}$ of what it is in oxygen-plenty and balanced breathing.

There may still be some doubt as to whether these low heats in extreme oxygen-want represent the heat production in *absolute* absence of oxygen,

but the fact that the *rate* of decrease has greatly diminished allows us to assume that the figures we have are very near the steady state of true anoxidative heat-production in smooth muscle. Let us for the present consider them as being such. The question then at once arises, what are the sources of this anoxidative heat?

There doubtless is heat from an elastic source. Calculation will show this to have a value much less than the observed anoxidative initial heat; therefore the latter fully covers the energy requirement of the tension rise.[3] This heat of elastic origin for the most part no doubt will be counterbalanced by the endothermic heat of the elastic recoil during the phase of falling tension.

In any event the mechanical manifestation of the contraction most probably has its prime origin in the acceleration of chemical reactions. Recent work has shown (6) that in smooth muscle, as in skeletal, lactic acid is liberated from the breakdown of glycogen when the muscle is stimulated to activity. If we assume that the amount liberated in smooth is the same as in skeletal muscle, then, from recent determinations of Meyerhof and Schulz (7) and as shown in the calculations,[4] this amount must be about 2.7×10^{-8} grams lactic acid per gram muscle per gram tension developed. The heat therefrom will be 2.68×10^{-8} (3836 − 3601), *or* 6.3×10^{-6} *gm.-cal.* Now since the initial heat in extreme oxygen-want is only 4.4×10^{-6} calorie it is obvious that either lactic acid formation during contraction is much less in smooth than in skeletal muscle, or that a large part of it is liberated after the contraction is over. Indeed when we consider the large proportion of the total anoxidative heat that is represented by the observed delayed heat in extreme oxygen-want, we find (if that heat is truly anoxidative) that there is no simpler way to account for it. For curiously the sum of the observed initial and delayed anoxidative heats, that is $(4.4 + 6.8)\ 10^{-6}$, or 11.2×10^{-6} *calorie*, is more than 75 per cent greater than the heat that would be liberated by the required lactic acid formation. The excess is accounted for partly by the probable resting metabolism of the muscle.

[3] The work done upon an elastic body by stretching is $W = \frac{1}{2} M \frac{e^2}{l}$, where M is Young's modulus (stated to be 95,000 for skeletal muscle in the older literature), e the change in length, and l the resting length, of the stretched body. For our smooth muscles the mean values for e and l may be put as 0.0009 cm. and 2.7 cm. respectively. Expressed in terms of gm. calories of heat we then have,

$$W = \frac{95000 \times 0.0009^2 \times 981}{2 \times 2.7 \times 4.19 \cdot 10^7} = 0.3 \cdot 10^{-6} \text{ gm. cal.}$$

as the work done by the muscle upon itself when exerting a tension of 1 gm. "isometrically."

[4] For example, the mean of 3 of their best experiments is 2.68×10^{-6} gm. lactic acid produced by 1 gm. gastrocnemius muscle exerting 1 gm. tension.

Upon subtracting the small anoxidative heats from the larger heats observed for the muscles in O_2-plenty we find large remainders for both the initial and delayed phases as is shown in the graph (fig. 1). This can only mean that for smooth muscle at least the oxidative processes, as well as the anoxidative, are distributed over both the major phases of the muscle action. For this reason in what follows let us abandon further separate consideration of initial and delayed heats. Let us rather add them together and consider the total heats produced during a single contraction.

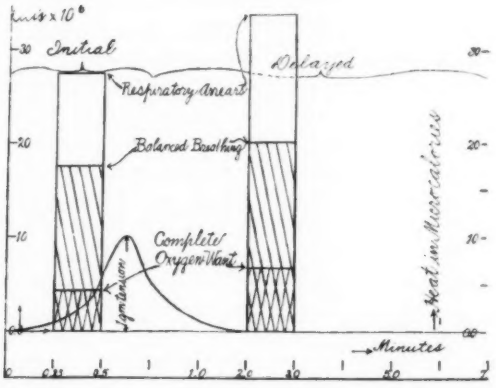

Fig. 1. Showing the mean heat production of smooth muscle during its response to nervous stimulation. The rectangles by their ordinates show the amounts of heat observed per gram muscle, per gram tension developed. The brackets in the diagram roughly indicate the time of the whole action over which the initial and delayed heats appeared. The positions of the rectangles represent roughly the points in time when the rates of production of these two heats were at a maximum; the areas of the rectangles have no significance.

In table 3 the sums of initial and delayed heats of muscles in balanced breathing are given, the sums being shown under the column numbered (1). This gives the total heat production during a whole muscle action in oxygen. In the next column under the number (2) we have put the nearest known and corresponding mean total anoxidative heat. In the last column under (3) is the difference between the figures under (1) and (2), or what, since that difference depends on the presence of O_2, we may designate heat of purely oxidative origin. What is the source of this oxidative heat?

Let us extend our assumption concerning the chemical reactions and assume that the mechanism of lactic acid removal in smooth muscle is the same as has been shown for skeletal muscle, and that the "oxidation

quotient" of this removal is equivalent to that determined lately for smooth, namely 1/3.35, by C. Lovatt Evans (6). Then for our smooth muscle 1/3.35 of the lactic acid liberated will be burned and this will produce 28.2×10^{-6} calories of heat $\left(\frac{1}{3.35} \times 2.68 \times 10^{-8} \times 3601 = 28.2 \times 10^{-6}\right.$ cal.). But along with the oxidation of the smaller portion there is a

TABLE 3

Summary of mean observations

Heat production per gm. smooth muscle per gm. tension in calories $\times 10^6$. (Microcalories)

EXPERIMENT	(V)	IN OXYGEN-PLENTY, BALANCED BREATHING			TOTAL ANOXIDATIVE	TOTAL OXIDATIVE
		"Initial"	"Delayed"	Total (1)	(2)	(3)
iv/11/28	5	15.0	22.7	37.7	11.2	26.2
iv/18/28	14	19.7	19.3	39.0	11.2	27.8
iv/ 4/29	5	17.6	28.6	46.2	17.3	28.9
v/16/29	7	19.6	15.8	35.4	9.2	26.2
v/16/29	5	20.3	17.2	37.2	9.2	28.3
Mean,	S = 36	17.4	20.1	38.9	11.6	27.5

Calculations: If 1 gram smooth muscle per 1 gram tension, as skeletal does, frees 2.68×10^{-8} gm. lactic acid and burns 1/3.35 during recovery then in terms of microcalories:

Heat of lactic acid liberation = 2.68×10^{-8} (3836–3601) =	+6.3
Heat of burning lac. acid = $1/3.35 \times 2.68 \times 10^{-8} \times 3601$ =	+28.2
Heat of lactic acid → glycogen =	− 4.5
Heat of oxidative origin remaining	+23.7
Total calculated heat production	+30.0
Total observed heat production	+38.9

resynthesis of $\frac{2.35}{3.35}$ of the total lactic acid, and this represents a negative heat of $1.88 \times 10^{-8} \times (3836 - 3601)$, or 4.5×10^{-6} calorie. The algebraic sum of these heats of opposite sign leaves $+23.7 \times 10^{-6}$ calorie, the calculated observable oxidative heat, if its origin is the oxidation of lactic acid or its carbohydrate equivalent. The mean of all the observed values for the total oxidative heats, as we may see in table 3, is somewhere about 27.5×10^{-6}, that is, about 16 per cent more heat observed than is demanded by theory. The agreement between calculated

and observed values thus is closer for the oxidative than for the anoxidative heats. (For summary of calculations, see appendix to table 3.)

Since the ratio Tl/H has been given the status of a constant for skeletal muscle contracting anaerobically (9), it may be of interest to know what the value of this ratio is for other muscles. This can not now be stated precisely for smooth muscle because no exact measurements of length were recorded. But from the limits of fixation of the muscles, which are roughly known, we can make a tentative calculation; from which it appears that the mean of greatly varying values of Tl/H for the smooth muscles is somewhere between 6.8 and 7.2, values which are nearer that calculated from the observations of Embden, Lehnartz and Hentschel (8). But this is true only if we take the *total* anaerobic heat of smooth muscle for the value of H in the equation. *If* we take the *initial* anaerobic heat for the value of H (and this was the original value used in the ratio for skeletal) then we have a value of the ratio equal to 18, if we include only the strictest anaerobic observations; and 14, if we include 6 observations where the anaerobiosis was not quite so complete. These higher values remind one of the ratio, 12 to 14, that Meyerhof (10) obtained on frog's gastrocnemius muscle.

Considering the result with the *total* heat, as value for H, we must conclude that, since the value is of the same magnitude as is found for frog's skeletal muscle the quantity of lactic acid liberated in smooth muscle is probably the same as it is in skeletal, assuming that lactic acid liberation is the source of the heat.

Turning again to the value of the Tl/H ratio when H is initial heat only, we are not yet forced to conclude, since the ratio now is much greater than that found for skeletal, that the efficiency of smooth muscle, therefore, must be greater than that of skeletal. For, if upon reinvestigation with sartorius muscle contracting in the absence of cyanide and in an $[O_2]$ so low that photobacteria cannot luminesce, it is shown that the initial heat of sartorius is as low as it is here shown to be for smooth muscle, then the value of the ratio, Tl/H, for skeletal muscle will also be higher, and the efficiency of both muscles more nearly the same.

CONCLUSION

From the foregoing it may be concluded: 1. That the true anoxidative heat can be determined better in a survival experiment by abandoning the use of cyanide and by inserting into the gas chain (preferably into the survival chamber itself, if feasible) a continuous indicator signaling the presence of oxygen whenever its concentration exceeds that of 0.001 per cent by volume at atmospheric pressure. In the present experiments photobacteria have proven to be useful as such an indicator.

2. That anoxidative heat in smooth muscle is distributed over both initial and delayed phases of its contraction, that the initial heat is more than sufficient to cover the energy requirements of the tension developed; but that to the observed initial a part of the delayed anoxidative heats is required to cover the heat production expected if its source is in the anaerobic breakdown of glycogen into lactic acid in the same amount as has been shown to be the case with frog's skeletal muscle. If this latter is the true source, then the delayed anoxidative heats in these experiments demonstrate that in smooth muscle a large part of the lactic acid is liberated *after* the period of rising tension is over, a view favoring that of Embden and his associates for skeletal muscle.

3. That the oxidative heat, like the anoxidative, is distributed over both initial and recovery phases of a smooth muscle contraction, and that the total purely oxidative heat of muscles in balanced breathing is nearly equal to that demanded by theory, if in smooth muscle the oxidative quotient is equal to 1/3.35.

4. That, if the carbohydrate metabolism as thus indicated is true for smooth muscle, then all other sources of heat, negative and positive, arising during contraction and recovery therefrom, must largely neutralize each other, and therefore need little further consideration in accounting for the final temperature changes observed during the muscle action.

5. The lengths of the muscles in this study were not exactly observed but from such data as are at hand as to length (l) the value of the ratio, Tl/H, does not appear to differ from that found for skeletal muscles in anaerobiosis, if the *total instead of the initial*, anoxidative heat is taken for the value of H.

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HORMONE CONTENT OF HUMAN OVARIAN TISSUES

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Relatively few attempts have been made to analyze human ovarian tissues for hormone content probably because of the small amount of normal tissue available. While searching for human tubal ova and studying the corresponding corpora lutea (1), (2), a considerable number of ovaries or parts of ovaries were obtained at operation. These were tested for hormone content by direct injections of fresh liquor folliculi or implants of tissue into ovariectomized rats. The full oestrous growth reaction of the vaginal epithelium as indicated by the cell content of smear preparations (3), (4) was used as a test of activity. The present series of experiments extends earlier analyses of extracts (5) and implants (6) of human ovarian tissues.

By injecting graded amounts of follicular fluid or implanting pieces of tissue graded as to weight, a roughly quantitative estimate of hormone content was obtained. The change in cell content (48 to 56 hours after injections or implants were made) from the control vaginal smear composed of leucocytes and varying numbers of epithelial cells to the oestrous smear composed only of cornified epithelial cells was considered a positive reaction. The least amount of tissue required to produce a full oestrous reaction was considered to contain 1 rat unit of hormone. Where some growth of the vaginal epithelium was indicated by an increase in epithelial cells, but the presence of a few leucocytes showed an incomplete reaction, the test was listed as indecisive (±). It is obvious that only small amounts of normal human ovarian tissue are obtained in the operating room and consequently only a limited number of tests of each specimen was possible. Therefore, such precise quantitative results as may be obtained in standardization of purified extracts were not practicable.

From the above description of the test used as a criterion of activity it will be understood that the hormone sought was that which produces growth in the genital tract and mammary glands. This growth results in the oestrous period in rodents (3), (7) and the growth phase of the men-

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strual cycle in primates (8), (9). Experimental menstruation in ovariectomized monkeys begins after the effects of this hormone wear off (8), (10). No attempts were made to test for an inhibitory substance such as that described by Papanicolaou (11), Kennedy (12), Parkes and Bellerby (13) and others, or for an active principle responsible for dissolution of the symphysis pubis of gophers (14) or relaxation of the pelvic ligaments of guinea pigs (15) as described by Hisaw; nor yet for a possible hormone which may be responsible for the premenstrual transformation of the primate endometrium or of the post-ovulation or pseudo-pregnant changes of some of the lower mammals as recently described by Corner and Allen (16) and by Weichert (17).

Wide variations in yields of this hormone from different ovarian tissues of large mammals have been obtained by different authors. In earlier analyses by means of the rat unit test the follicle was stressed as the principle source. The concentration in corpora lutea of lower mammals was found to be very low. The human ovary differs somewhat in that this active substance is present in the corpus luteum (5), obviously as a continuation of the secretory function of the follicle, for a considerable time after ovulation. Large amounts are also found in the placentas of certain animals (4, p. 631), (18, p. 228) although for some as yet unexplained reason the placentas of other animals contain little or none of this material. Just how this hormone may be related to the substances mentioned in the preceding paragraph is not altogether clear.

Two groups of investigators have reported yields from "residual ovarian tissue" of pigs quite comparable to those from follicular fluid (19), (20). The method of separation has been to aspirate the follicular fluid or to enucleate frozen masses of it from refrigerated ovaries. Such procedures of course leave the follicular epithelium in place. Consequently, the residual ovarian tissue is partly follicular and can by no means be considered ovarian stroma.

Zondek and Aschheim (6) have reported positive results from implants of corpora lutea and ovarian interstitial tissue from human ovaries.

Another major necessity for continuing analyses of isolated ovarian tissues arises from the recent experiments of Parkes (21) upon mice sterilized by x-rays. By carefully regulated exposures he has been able to destroy ovarian follicles without apparently injuring the hormonal function of the ovaries, for the oestrous rhythm was not interrupted. The transformations of these ovaries have been followed by Brambell, Parkes and Fielding (22) in histological studies which show that a proliferation of the germinal epithelium of the ovary occurs which forms epithelial ingrowths, similar to earlier embryonic ones, but free from ova. While this tissue is apparently not follicular, it is obviously not equivalent to normal ovarian stroma. The conclusion reached is that the follicles are

not necessary for continued secretion of the ovarian hormone which is responsible for oestrus. Several writers have inferred from this that perhaps ovarian stroma or "interstitial tissue" of the normal ovary may share ovarian endocrine function. Consequently additional analyses of isolated normal ovarian tissues are desirable.

Our first objective in this investigation was to obtain quantitative analyses of hormone content of the corpora lutea corresponding to human ova recovered from the uterine tubes and this was extended to include a considerable series of corpora lutea at different periods of the menstrual cycle and a few during pregnancy. The second objective was a continuation of analyses of the follicles, both the liquor folliculi and pieces of the follicle wall. In many cases ova were recovered from those follicles and sectioned, so that a diagnosis of normality or atresia was possible (23). This is much more necessary in the human ovary than in those of litter bearing animals, for in the former a single follicle of the many larger ones present in both ovaries is destined for ovulation, while in the latter from 7 to 10 or 12 large normal follicles may be present at one time.

The third general class of tissues analyzed included connective tissue of the ovarian cortex which did not contain follicles large enough to be visible to the naked eye. In all cases these pieces of stroma from the ovarian cortex were clipped from ovaries which contained corpora lutea or large follicles which returned positive tests for the presence of hormone. Therefore other parts of these same ovaries contained this hormone in considerable concentration. It is thought that this tissue makes a clear separation between the follicles, corpora lutea and ovarian stroma as to hormone function.

All tests were made of fresh tissue as soon as possible after removal at operation. The recent menstrual history was known so that the data could be oriented with regard to the menstrual cycle.

In addition to the tissue listed above opportunity has arisen to test certain pathological ovarian tissues; cystic follicles or corpus luteum cysts which have been related with histories of irregular or abnormal menses.

The test animals were young adult rats from which the ovaries had been removed two weeks prior to the first test. The first test consisted of "priming" injections of two rat units of purified extract into all animals. For subsequent tests each animal was used no oftener than once a week and if two successive negative tests were recorded for the same animal, it was given an experimental oestrus by means of injections of extract before a third test. With careful standardization of the technique of the rat test (24), (25) there is little chance of such extremely divergent results as those reported by Coward and Burn (26).

The ovarian tissue tested was used as soon as possible after removal

from the patient, the tissues being refrigerated when it was necessary to delay this for one or two hours. Where liquor folliculi was used no attempts were made to dilute the smaller amounts up to a uniform volume. In case of the cellular tissues the whole structure was weighed and a series of pieces of different sizes clipped off and weighed, so that hormone yield per gram of tissue might be estimated.

Implants were made under aseptic precautions in subcutaneous pockets. The larger pieces were cut into small portions to make absorption of the hormone from the tissue more uniform and complete. Where considerable amounts of tissue were implanted, subcutaneous lumps often resulted. There was very little trouble from infection or sloughing. Before the second test in the same animal the larger lumps were dissected free and removed. In most cases they were definitely encapsulated with connective tissue from the host.

OBSERVATIONS. *Hormone content of recent corpora lutea, three of which correspond to unfertilized ova recovered from the uterine tubes.* The gross and microscopic description of these corpora has been given in detail in the paper describing the recovery of tubal ova (1). A brief summary would stress the following principal points. In the recent specimens the rupture points were still open, usually being filled with small gelatinous plugs. In size the corpora seldom exceeded 20 mm. in greatest diameter. In some cases, where the corpus had not been fully distended, smaller diameters were considerably less than 20 mm. In weight these corpora ranged from 0.52 gram to 1.77 gram. When these recent corpora were opened by cuts through the rupture points they were usually found to be in a hemorrhagic stage. Often the center was filled with a gelatinous substance surrounded by an area of hemorrhage adjacent to the luteal cells. The luteal walls had not developed the yellow pigment typical of older corpora. The central cavities of several contained fluid, indicating an early stage of transition from follicle to corpus luteum.

Microscopically the rupture point in most cases was found to be open, with varying degrees of cell ingrowth beginning to heal the gap. Material in the central cavity after fixation of the corpus usually showed a reticular coagulum containing varying numbers of wandering cells. The luteal walls showed beginning transformation from the follicle cell to the luteal cell type and varying degrees of migration of fibroblasts from the theca interna through the lutein layers to encapsulate the material in the central cavity.

On cutting open the corpus luteum the material in the central cavity, if gelatinous, usually adhered to the wall and in cutting small portions for implants a part of each was usually included. In the earlier experiments fairly large pieces of tissue were implanted, sometimes as much as a gram of tissue being included in one implant. After the first few tests

TABLE 1

Hormone content of human corpora lutea in the absence of pregnancy

Several of these corpora correspond to ova recovered from the uterine tubes.

CORPUS LUTEUM	DAY OF MENSTRUAL CYCLE	WEIGHT OF CORPUS	TISSUE IMPLANTED, WEIGHT	RESULTS OF TESTS	TOTAL RAT UNITS OF HORMONE	RAT UNITS PER GRAM	REMARKS
		grams	*grams*				
23B	13th	>1	0.5	+			Recent ovulation: fluid in center of corpus set in gel after removal
			0.2	+			
			0.1	+			
			0.07	+	>14.0	>14	
			0.05	−		<20	
18A	14th	0.95	0.24	+			Recent ovulation
			0.11	+	>8.5	>9	
			0.065	−		<15	
9A	14th	1.17	0.58	+			9A and 10A are two corpora from the same ovary; 9A probably 1 month old
			0.29	+	>4.0	>3.5	
			0.14	−		<7	
10A	14th	0.52	0.26	+			Corpus 10A from a recent ovulation
			0.13	+			
			0.06	+	>8.6	>16	
2P	No record	1.05	0.06	+	>17.5	>16	Open rupture point, fluid in cavity
17A	15th	1.77	0.4	+			Corresponds to tubal ovum I, recent ovulation
			0.21	+			
			0.08	+	>22	>12.5	
			0.06	±		<17	
			0.04	−			
25Br	15th		0.21	+			Corresponds to ovum II, one of twins
			0.07	+		>14	
25Bl	15th		0.23	+			Corresponds to ovum III, the other twin
			0.07	+		>14	
11A	16th	1.05	0.52	+			Corpora at same stage of development in same ovary
			0.26	+			
			0.13	+	>8	>7.5	
12A	16th	1.57	0.78	±			Probably twin ovulations, 11A and 12A
			0.39	+			
			0.19	+	>8	>5	
1P	17th	1.65	0.07	+	>23	>14	

TABLE 1—*Concluded*

CORPUS LUTEUM	DAY OF MENSTRUAL CYCLE	WEIGHT OF CORPUS	TISSUE IMPLANTED, WEIGHT	RESULTS OF TESTS	TOTAL RAT UNITS OF HORMONE	RAT UNITS PER GRAM	REMARKS
		grams	*grams*				
16A	20th	1.4	0.70	+			
			0.35	+	>4	>3	
			0.17	−		<6	
15A	22nd	1.57	0.78	±			
			0.39	+	>4	>2.5	
			0.19	−		<5	
			0.09	−			

it was found that positive reactions were usually obtained from less than 0.5 gram of recent corpora.

The most important details of the tests of normal corpora lutea in the absence of pregnancy are included in table 1. The specimens are listed according to time sequence in the menstrual cycle. Three of these corpora (17A, 25Br, and 25Bl) correspond to unfertilized ova recovered from the Fallopian tubes. Several others were from recent ovulations in cases where searches for corresponding ova were unsuccessful or where definite identification of tubal specimens after sectioning was not made.

The times in the menstrual cycle at which the corpora were removed are listed in column 2. Ovulation, as checked by recovery and definite identification of five tubal ova, occurs on or shortly before the fourteenth (morning of the fifteenth) day following onset of the previous menses, perhaps as early as the twelfth day (1). Corpora dated between the thirteenth and seventeenth days therefore represent recent ovulations. Two older corpora obtained on the twentieth and twenty-second days of the menstrual cycle respectively are included. Of special interest also are the two corpora (9A and 10A) from one ovary removed on the fourteenth day of the cycle, one of which was recent and the other older. After a histologic study of these, one was estimated as very recent and the other as at about a month's development. The former contained more than 16 rat units of hormone per gram, the latter still contained more than 3.5 rat units per gram of tissue.

The highest yield of hormone by this implant method was more than 16 rat units per gram of luteal tissue (10A). Yields of more than 12.5, 14 and 14 rat units per gram respectively were obtained from the three corpora corresponding to tubal ova I, II and III (17A, 25Br, 25Bl). In several cases the implants were not graded down in weight to the point where negative results were obtained from the smallest pieces because of difficulty of handling such small pieces and the belief that a wide range of

error where dilution was not feasible might invalidate the results. Yields are expressed in terms of more than the minimum. In cases where tests at the "small end" of a graded series were negative, the possible range may be calculated.

In a total of thirty-eight tests in this series only three were indeterminate (±). One of these (17A) resulted from an implant of 0.06 gram while 0.08 gram was positive and 0.04 negative. This might therefore be expected. The other two (12A, 15A) were from larger implants than resulted in positive tests further down in the series and are therefore irregular. The majority of these tests, however, are as clear-cut as could be expected for such a "biological test unit."

The total rat unit of hormone per corpus luteum is interesting. The greatest amounts present in the largest corpora (17A and 1P; 1.77 and 1.65 gm.) are more than 22 and 23 rat units respectively. If the content of both corpora corresponding to twin ova II and III are added, a total of more than 28 rat units would represent the hormone present in the recent luteal tissue of the two ovaries of this patient on the 15th day of the menstrual cycle.

The smaller yields from corpora obtained on the 20th and 22nd days of the cycle (16A and 15A) would indicate a considerable decrease in hormone content with an increased age (interval following ovulation).

The results from corpus 9A, estimated at about one month following ovulation, indicate the presence of some hormone. Others estimated at this age have given negative tests. More data from additional tests of corpora of these stages (identified histologically) are needed.

Several tests were made of older corpora (from non-pregnant cases) in which the yellow "luteal" pigment was well developed and the contents of the central cavity well encapsulated by connective tissue. These tests were uniformly negative.

It should be borne in mind in a consideration of these results that the recent corpus is really a transition structure from the ruptured follicle and is composed mostly of transforming follicular cells and filled with a fluid or gelatinous substance made up partly of continued secretion from these cells somewhat similar to follicular fluid.

Hormone content of corpora lutea of pregnancy. In table 2 are listed the most important details of tests of three corpora of early pregnancy and two of full term. The latter were obtained at caesarian operations at which normal babies were delivered. Of the former, one was from an ectopic pregnancy of two to three months' standing, a second was a normal corpus of the second month corresponding to an 8 mm. embryo with membranes intact which was removed from a myomatous uterus, and a third was from a case in which an empty chorionic vesicle, still intact and apparently fairly normal, was removed from the uterus. In the last case

(13A) the corpus was cystic, containing 17.5 cc. of clear straw-colored fluid,—therefore obviously very abnormal. The appearance of this cystic corpus was very interesting. One-third of the surface was yellow luteal tissue,—the rest resembled the wall of a large follicle and this part seemed to rest in the luteal part like an acorn in its cup. Histological section showed this to be one structure. The fluid only was tested for hormone content. It was quite toxic in the larger doses. Two indecisive tests in larger amounts might possibly be due to this effect. Tests were not carried far in graded series, consequently the estimate of more than 35 rat

TABLE 2

Hormone content of human corpora lutea of pregnancy

CORPUS LUTEUM	STAGE OF GESTATION	WEIGHT OF CORPUS	WEIGHT OR VOLUME OF TISSUE IMPLANTED	RESULTS OF TESTS	TOTAL RAT UNITS OF HORMONE	RAT UNITS PER GRAM	REMARKS
		grams	*cc.*				
13A*	2-3 months	†	2.0	+			Cyst wall not implanted; specimen obviously abnormal
			1.5	±			
			1.0	±			
			0.75	+			
			0.50	+	>35	>2	
			grams				
15B	2nd month	1.56	0.66	±			8 mm. embryo in amnion 18.5 × 16.5 × 9.5 mm.
			0.49	+			
			0.25	+			
			0.16	+	>9.7	>6.6	
3P	2-3 months	0.825	0.08	+	>10	>12.5	Ectopic pregnancy
1A	Full term	1.0	1.0	−	<1	<1	Caesarean section
4A	Full term	1.0	1.0	−	<1	<1	Caesarean section

* Fluid from cystic corpus.

† Containing 17.5 cc. of fluid.

units of hormone in this corpus, exclusive of the wall, represents a small part of the total. One wonders if perhaps this abnormal corpus might have been related to the death of the embryo.

It is probable that higher yields would have been obtained in tests of corpora 15B and 3P if it had been possible to extend these experiments. The reason for this limitation was that we were working with fresh tissues and a limited number of test animals.

In contrast to these relatively high yields from corpora lutea of early pregnancy are the two negative results from large implants (1 gm. each)

of the two full term corpora (1A and 4A). These corpora were typical full term specimens, the luteal-cell walls being orange yellow and the centers white connective tissue.

These negative results are very interesting because of the great quantities of this hormone in the human placenta, blood and urine during the latter half of pregnancy. They show definitely that the corpus luteum of pregnancy no longer shares the secretion of this hormone in late gestation in woman.

Yields from liquor folliculi, follicle walls and cortical stroma without macroscopic follicles. Most of these specimens were removed between the 11th and 18th days of the menstrual cycle. In a majority of cases the follicle chosen for analysis was the one which was considered the most likely for the next ovulation, which might have been expected at some time between the 12th and 14th days.

The follicles were dissected free from the stroma and measured. At this stage they resembled white grapes after removal of the skin,—although somewhat more transparent. Often, with the use of a strong light, the ovum could be located in the intact follicle. Then the wall of the follicle was slit open and the ovum recovered, embedded and sectioned in order that the normality or atresia of the follicle might be diagnosed. We also hoped to secure normal human ova which had begun maturation division (23).

Then the liquor folliculi was collected in a hypodermic syringe and the follicle walls cut up and weighed for implants.

A description of some of the most interesting ova obtained in this work is given in another paper (23). One of the most important findings was that many of the large follicles visible on the surface of the human ovary were not normal, although from a gross appearance they were often indistinguishable from normal follicles. This point should be kept in mind in a consideration of yields of hormone from human ovaries.

The pre-ovulation size of follicles in the human ovary is still doubtful. From measurements in other animals one would expect it to be less than that of the fully developed corpus luteum. It is usual to consider follicles larger than 15 mm. in average diameter to be cystic. It was an occasion for considerable surprise therefore to obtain several follicles about 20 mm. in average diameter which still appeared normal,—containing normal ova and proliferating follicle cells as indicated by a high incidence of mitotic division. These few may have been exceptional for several others of these larger follicles as judged from the condition of the ova, had already begun atresia. It seems quite probable, however, that the pre-ovulation size of the human follicle may reach from 10 to 15 mm.

In several of the later cases tests of the liquor folliculi (L.F.) and follicle walls (F.W.) of the same specimen were made in order to arrive at a total

hormone content. Where this was done the two are placed in adjacent positions in table 3.

In several cases the doses of liquor folliculi injected were not graded down to the point where negative tests resulted. Consequently the yields

TABLE 3

Hormone analyses of fresh ovarian tissues: liquor folliculi (L. F.) by injections, follicle walls (F. W.) by implants

SPECIMEN NUMBER	DAY OF CYCLE	SIZE OF FOLLICLE (DIAMETER)	CONDITION OF OVUM OR FOLLICLE	TOTAL VOLUME OR WEIGHT	AMOUNTS TESTED	RESULTS OF TESTS	TOTAL RAT UNITS	RAT UNITS PER CUBIC CENTIMETER OR GRAM
L.F.5	11th	28 x 18 x 16 mm.		1.8 cc.	1.2 cc. 0.6 cc.	+ ±	>1.5	>0.8
F.W.4	12th	20 x 20 x 16 mm.	Normal	0.77 gm.	0.47 gm. 0.18 gm.	+ +	>4	>5
L.F.14		From same follicle		1.87 cc.	1.0 cc. 0.5 cc. 0.37 cc.	+ + +	>5	>3
L.F.7	13th	24 x 22 x 19 mm.		3.4 cc.	1.5 cc. 1.0 cc. 0.60 cc. 0.40 cc.	+ + + ±	>5	>1.5
F.W.2	13th	27 x 24 x 18 mm.	Early atresia	0.9 gm.	0.45 gm. 0.22 gm.	+ +	>4	>4.5
L.F.12		From same follicle		2.8 cc.	1.5 cc. 0.8 cc. 0.5 cc.	+ + +	>5.6	>2.0
F.W.3	13th	25 x 22 x 20 mm.	Atretic	0.67 gm.	0.67 gm.	+	>1	>1.5
L.F.13		From same follicle		4.2 cc.	1.55 cc. 1.0 cc. 0.5 cc. 0.33 cc. 0.25 cc.	+ + ± − ±	>4.2	>1
L.F.2	14th	11 x 11 x 9 mm.	Atretic	1.65 cc.	1.0 cc. 0.65 cc.	− −		<1
L.F.3	Same ovary	11 x 11 x 11 mm.		0.62 cc.	0.62 cc.	−		
F.W.1	16th	8 small follicles		>0.5 gm.	>0.5 gm.	+		>2
L.F.1	18th	Very large	Cystic	3.0 cc.	1.0 cc. 0.6 cc. 0.4 cc. 0.25 cc.	+ + − −	>5.0	>1.6 <2.5

of hormone as listed are considerably under the possible totals. Furthermore a whole dose of liquor folliculi was given in a single injection, instead of in three or more injections spaced over a considerable interval, as used in standardization of extracts. This may have increased slightly the

amount of hormone per rat unit. We hope to continue this investigation using smaller amounts of liquor folliculi, for it is obvious from a study of table 3 that in the majority of cases the tissues tested probably contained more hormone than indicated by the figures. The highest yield from implants of follicle walls was 5 rat units of hormone per gram (F.W.4), that for liquor folliculi 3 rat units per cc. (L.F.14). The highest total for any one follicle, including both wall and liquor, was 9 rat units from a follicle removed on the 12th day of the menstrual cycle. Neither of these tests was graded down to the point of negative reactions.

It is noteworthy that all specimens listed in table 3 were dissected from ovaries which contained no recent corpora lutea. The second case listed on the 13th day of the menstrual cycle (F.W.2 and L.F.12) had no corpora at all in either ovary and yet had a history of several fairly regular menstrual periods.

TABLE 4

Tests of cortical stroma without follicles visible macroscopically

SPECIMEN	DAY OF MENSTRUAL CYCLE	REMARKS	TOTAL WEIGHT OF TISSUE	TISSUE IMPLANTED	RESULTS OF TESTS
			grams	*grams*	
C.S.1	14th	C.L.18, containing more than 9 R.U. of hormone, in this ovary	0.89	0.89	–
C.S.2	1st	This ovary contained 7 corpora lutea	1.07	1.07	–
C.S.3	15th	From ovaries which produced twin ova 2 and 3; each corpus contained more than 14 R.U. of hormone	2.65	1.65 1.0	– –

Four tests (listed in table 4) were made of cortical stroma which did not contain follicles large enough to be visible to the naked eye The ovaries from which these specimens were clipped also had in them either follicles or corpora lutea which were found to contain considerable amounts of hormone. Consequently these results are not open to the criticism that the ovary may have been inactive as to internal secretion. In these four tests relatively large implants were made. All results were negative. Compare for instance the implants of 1.65 and 1.0 grams of stroma (C.S.3) with the implants of pieces of corpus luteum from these same ovaries (corpora lutea 25Br and 25Bl, table 1). These latter gave positive results in pieces weighing only 0.07 gram. The ovaries from which both were dissected were those of the case from which the twin ova were recovered from the tubes. These data although meager indicate rather a clear-cut localization of tissue concerned with secretion of this hormone.

Hormone content of ovarian tissue in cases with abnormal menstrual histories. Under this head are included one case of amenorrhea and two cases of long continued bleeding (table 5). In the former case the patient had not menstruated for three months. The ovaries were cystic and contained no corpora lutea. From several of the larger follicular cysts 7.3 cc. of clear straw-colored fluid was obtained. This was very similar in appearance to normal liquor folliculi. It was negative in 5 tests, the largest amount tested being a 2 cc. injection.

In both cases of long continued bleeding considerable amounts of hormone were present. In the first (L.F.11) the tests indicate more than a total of 19 rat units in the cystic fluid alone and the tests were not carried

TABLE 5

Hormone content of ovarian tissues from cases with abnormal menstrual histories; liquor folliculi (L. F.) and follicle walls (F. W.)

SPECIMEN NUMBER	TYPE OF IRREGULARITY	SIZE OF FOLLICLE (DIAMETER)	CONDITION OF OVUM OR FOLLICLE	TOTAL VOLUME OR WEIGHT	AMOUNTS TESTED	RESULTS OF TESTS	TOTAL RAT UNITS	RAT UNITS PER CC. OR GM.
L.F.11	Long continued hemorrhage	2 cystic follicles 30 x 25 x 20 mm.	Cystic; (no corpora lutea)	9.6 cc.	2.0 cc. 1.0 cc. 0.5 cc.	+ + +	>19	>2
F.W.5	Long continued hemorrhage	30 x 30 x 30 mm. and several small follicles	Cystic, (no corpora in either ovary)	1.05 gm.	0.59 gm. 0.3 gm.	+ +	>3	>3
L.F.15				14.0 cc.	2.0 cc. 1.5 cc. 1.0 cc. 0.5 cc. 0.33 cc.	+ + + + +	>42	>3
L.F.8B	3 months amenorrhea	26.5 x 23 mm. and several smaller	Atretic, (no corpora lutea)	7.3 cc.	2.0 cc. 1.0 cc. 0.7 cc. 0.5 cc. 0.2 cc.	− − − − −	<3.7	<0.5

to the point of negative reactions. In the second case there was a total of more than 42 rat units of hormone present in the cystic fluid and considerable amounts in the walls of the cysts. In neither of these cases was the fresh cystic fluid toxic.

Effect of removal of ovaries or of excision of corpora lutea or large follicles upon the appearance of the next menses. There are several reports in the clinical literature of cases where removal of recent corpora lutea or of ovaries containing active corpora lutea was followed by menstruation in less than forty-eight hours following operation (Pratt, 27). Additional evidence of the same sort in monkeys has been described by Allen (1), (28). In the latter paper it has also been demonstrated in monkeys that injury to large follicles in ovaries which contained no corpora lutea, if the

operation was performed in the intermenstruum, also resulted in the onset of menses within forty-eight hours.

During the course of this present work in several cases where ovaries were normal it has been possible to observe the effect of excision of the most recent corpus on the appearance of the next menses. Whenever this was done between the 12th and 18th days of the cycle menstruation began on the following day or the second day following operation.

In our search for maturation divisions in human ova several large follicles have been excised from normal ovaries in cases where there had been no recent ovulation. In most of these the operation was dated during the intermenstruum. The following case is described in detail as typical of this effect. This patient was operated on the 18th day of the menstrual cycle (dated from the onset of the previous menses). One ovary appeared normal; the other was somewhat enlarged and contained an old corpus luteum (there had been no recent ovulation), several large follicles and a "chocolate cyst." This ovary was removed. From the large follicles 3 cc. of clear straw-colored liquor folliculi (L.F. no. 1, table 3) were aspirated. This contained more than five rat units of hormone. The "chocolate cyst" was considered from its general appearance to be inactive as far as participation in endocrine function was concerned. Menstruation began on the day following operation.

Five other cases of removal of a recent corpus luteum or a large follicle with similar results have been encountered.

It seems probable therefore that removal during the intermenstruum of part of either ovary most active in hormone production (either large follicle or recent corpus luteum) precipitates menstruation within the next two days.

Discussion. The question of the distribution of hormone in ovarian tissues is one which requires an appreciation of anatomical differences in ovaries of different mammals for full understanding. The adult human ovary, when compared to those of other animals, is noteworthy for the scarcity of small follicles. For this reason a much more complete separation of the human ovary into constituent tissues for hormone analysis is possible. This is especially true for ovarian stroma which may be obtained relatively free from small follicles.

Also the human corpus luteum is quite different structurally from the corpus luteum of some of the other mammals. It retains a central cavity filled with either a fluid or gelatinous substance which becomes encapsulated or partially walled off by connective tissue growth. The usual hemorrhage into the central cavity of the corpus of woman is seldom found in corpora of lower mammals where the luteal cells and invading cells of the former theca interna completely fill in the cavity of the former follicle. It is quite possible that this difference is significant as regards endocrine activity.

Some of the earlier investigators stressed the importance of the corpus luteum of the pig and cow and reported it to be the most active endocrine tissue. In making extracts the follicular part of the ovary was often discarded. Later it was shown that in these animals the corpus luteum contains very little of this hormone, most of the active substance being located in the follicles. The human corpus luteum, however, contains considerable amounts. It was also found in the pig that recent corpora containing fluid in the central cavities often gave positive tests (3). Parkes and Bellerby (18) report similar findings. The most logical interpretation seems to be that this is a transition from the activity of the follicle. It appears therefore that following the first three or four days after ovulation, when the corpora of the pig and cow have become solid tissue masses, they contain negligible amounts of this hormone.

It will be noted from the study of table 1 that all human corpora lutea removed between the 13th day and end of the menstrual cycle gave positive results. This would indicate that for at least two weeks the human corpus contains this hormone. Corpora removed during the first two weeks of the menstrual cycle, which were clearly the result of an ovulation from two to four weeks previously, sometimes returned positive, at other times negative tests. Although our data are meager, a smaller yield with increasing age of the corpus is indicated.

The objection may be raised that the presence of hormone in this tissue is not necessarily indicative of secretion. It is quite possible that with the ingrowth of the connective tissue from the theca through the former granulosa cells to encapsulate the contents of the central cavity that a diminution or cessation of secretion occurs. The presence of this particular hormone in the corpus luteum seems to be clearly a continuation of a function of the follicle. In the pig and cow the period is a matter of a few days, in non-pregnant woman, of a few weeks. Just how it may be related to other active substances extracted from corpora of lower mammals is problematical.

In earlier papers high yields of hormone from corpora lutea during the first half of pregnancy have been reported. It is interesting to note from tests of two corpora obtained at caesarian section that the full term corpus contains very little if any of this active principle. It has already been demonstrated that very great amounts are present in the placenta, in the circulating blood and in the urine (4). This might be interpreted as indicating that the placenta has taken over this endocrine function begun by the follicle and (in woman) continued by the corpus luteum. This is especially interesting as concerns the source of the hormone responsible for development of the mammary glands during late gestation (18). It seems important that quantitative analyses of corpora lutea of the latter part of gestation be made whenever possible in order that further information

concerning the cessation of secretion of this hormone by the corpus of pregnancy may be obtained. Its presence in placentas of some mammals and absence from others offers an interesting field for further investigation.

The positive results from tests of fluid from cystic follicles in the two cases of long continued bleeding are interesting. They are difficult to correlate with experimental menstrual periods in monkeys (8), (9), (10). In these experiments menstruation followed removal of ovaries and cessation of hormone administration.

From the effects observed after excision of large follicles and recent corpora lutea it seems probable that conclusions as to the cause of menstruation drawn from experimental work in monkeys hold good for woman; i.e., that after the ovarian hormone responsible for growth in the genital tissues has been acting for a period, if this influence be removed by excision of the most active ovary or part (large follicle or recent corpus luteum) or, in experimental cycles in monkeys, by cessation of injections of hormone, menstruation will follow within the next few days. The interpretation would be that menstruation is degenerative in significance, equivalent to the metoestrum of lower mammals, and due primarily to decreased secretion of this ovarian hormone.

SUMMARY AND CONCLUSIONS

1. Analyses of hormone content of isolated tissues of the human ovary have been made by injections of fresh follicular fluid and by implantation of fresh corpora lutea, of follicle walls and of cortical stroma free from macroscopic follicles. The use of graded amounts of ovarian tissues has made possible roughly quantitative analyses.

2. The test used as a criterion of hormone activity was the full oestrous reaction of the ovariectomized rat as reflected in the cell content of the vagina. Therefore the hormone sought was that responsible for the genital growth reactions of the periods of oestrus in lower mammals and of the intermenstruum in primates.

3. Three of the recent corpora lutea tested corresponded to unfertilized ova recovered from the uterine tubes. The stage of development of these and other corpora has been checked by histologic studies. High yields of this hormone have been obtained from recent corpora removed from the 13th to 17th days of the menstrual cycle. There is a considerable decrease in amount by the 20th to 22nd day. Corpora of the first third of gestation contain considerable amounts. Two full term corpora gave negative tests. This last result indicates definitely that toward the end of gestation the corpus luteum of woman does not share responsibility for the large amounts of this hormone in the placenta, blood and urine.

4. Liquor folliculi and pieces of follicle wall (mostly granulosa cells) contained considerable amounts of this hormone. In most cases the

specimens tested were diagnosed as to normality or atresia by study (in section) of the follicle walls or the contained ova. In two cases of long continued bleeding large yields of hormone were obtained from cystic follicles. In one case of amenorrhea, large amounts of fluid from cystic follicles returned negative tests.

5. Cortical stroma without macroscopic follicles, taken from ovaries containing follicles or corpora in which hormone was demonstrated, gave negative tests.

6. These results added to earlier data demonstrate that, as far as the secretion of this hormone is concerned, the human corpus luteum merely continues an activity which is primarily follicular. This hormone content of the human corpus is highest just after ovulation in the early stages of transition from follicle to corpus luteum and decreases before the onset of the next menses. Normal stroma tissue of the ovarian cortex shares little in this function.

7. Removal of parts of ovaries most active in secretion of this hormone, either large follicles or recent corpora lutea,—when done during the intermenstruum, usually results in the appearance of menstruation within the next forty-eight hours. These observations substantiate earlier conclusions from experimental work in monkeys that menstruation is degenerative in significance and primarily due to decreased action of this ovarian hormone after it has induced a certain amount of growth in the uterus.

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THE ACTION OF INSULIN ON THE STORAGE AND UTILIZATION OF SUGAR BY THE ISOLATED NORMAL AND DIABETIC HEART

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While it is accepted that the liver and the musculature of the body play the most prominent part in sugar metabolism, and while a final analysis of the action of insulin will demand a consideration of the body as a whole, the action of isolated organs in their full functional capacity still demands investigation. To limit the bounds of the investigation and to eliminate as many associated factors as is compatible with the investigation of such a complex affair as sugar metabolism it was decided to look at the problem only in so far as the heart was concerned. It must always be borne in mind that results of experiments upon isolated organs, however illuminating they may be, can never be accepted as wholly indicative of the function of the organ in the whole animal.

Numerous experiments have been performed and the results especially for sugar utilization and muscle sugar have been strikingly uniform. At first difficulty was encountered with glycogen estimations, but by using not more than 3 to 4 grams of muscle satisfactory duplicates were ultimately obtained.

The purpose of the work was to make a comparative study in isolated normal and diabetic hearts, with and without the addition of insulin to the perfusing blood, of the glycogen, muscle sugar and sugar utilization of the heart at high, normal and low levels of sugar concentration. The effects produced by low levels of sugar concentration and by progressive hypoglycemia will be dealt with in a later paper.

METHODS. *Blood sugar:* This was estimated by MacLean's method.

Glycogen: This was estimated by the Pflüger method using 30 per cent KOH for digestion and absolute alcohol for precipitation with a final dilution of approximately 75 per cent. Precipitation was completely effected by centrifuging after allowing the solution to stand overnight. The precipitate and the residue on filter paper were dissolved in water, the solution was neutralized with acetic acid and 1.1 cc. of conc. HCl added to every 16 cc. of solution present.

Muscle sugar: A piece of muscle about 5 grams in weight was weighed

in a weighing bottle as in the estimation of glycogen. It was ground in a mortar containing purified sand and about 5 cc. of alcohol, then set aside in the ice chest for about one hour after which it was again ground and transferred to a beaker by washing with about 40 cc. of alcohol to precipitate the glycogen. The beaker was covered with a watch glass and set aside in the ice cupboard for 2 to 3 hours. The contents of the beaker were

TABLE 1

The glycogen content of the normal and diabetic heart

Normal

HEART FROM WHOLE ANIMAL		HEART FROM HEART-LUNG PREPARATION		DURATION OF EXPERIMENT
R. V.	L. V.	R. V.	L. V.	
				hours
0.557	0.476	0.669	0.470	2
0.737	0.558	0.590	0.367	2
0.631	0.625	0.421	0.646	3
0.583	0.680	0.584	0.533	4
0.631	0.679	0.551	0.494	2
0.386	0.427			
0.736	0.568			
Average 0.609	0.573	0.563	0.502	2½

Diabetic

HEART FROM WHOLE ANIMAL		HEART FROM HEART-LUNG PREPARATION		DURATION OF EXPERIMENT	DURATION OF DIABETES
R. V.	L. V.	R. V.	L. V.		
				hours	*days*
1.100	1.181	0.875	0.860	3	5
0.608	0.677	0.671	0.651	3	5
0.890	0.700	0.890	0.760	2	4
		0.806	0.972	3	3
		0.525	0.410	4	5
Average 0.866	0.852	0.753	0.730	3	4½

then filtered through a starch free filter paper the residue on the filter paper and the deposit in the beaker were carefully washed several times with small quantities of alcohol, and the washings filtered and added to the main filtrate. The filtrate was concentrated to about 2 cc. in vacuo and at a temperature of 40°C. to 50°C. After concentration the remaining bulk of the liquid was transferred to a 50 cc. stoppered measuring cylinder. The flask in which the liquid had been concentrated was washed with water and

the washings added to the cylinder. This brought the volume of the liquid to about 25 to 30 cc. One-third of this volume of N/10 sulphuric acid was added to make the acidity of the liquid in the cylinder equivalent to N/40 H_2SO_4. Lloyd's reagent (1.5 grams for every 25 cc. of the liquid) was then added to the cylinder and the whole thoroughly shaken, and filtered into a 50 cc. volumetric flask. The cylinder and the residue on the filter paper were washed by means of water and the washings also filtered into the 50 cc. flask. The volume of the filtrate was made up to 50 cc. and sugar was estimated by MacLean's method.

Depancreatization: In order to obtain thoroughly diabetic animals a complete pancreatectomy was performed. We have found that the best way to remove the pancreas in the dog is first to remove the head, carefully separating it from its connection with the superior mesenteric vein, then, by blunt dissection and by using dry swabs, to break through the peritoneum binding the neck to the pyloric region thus freeing the gland from the gastro-duodenal artery and its two branches, the right gastro-epiploic artery and the superior pancreatico-duodenal artery. If this be not done at the beginning but the easier and more accessible portions taken first, then congestion and its attendant ills will considerably delay the operation and tend to give bad post-operative results. Much is ultimately gained in good after results by the rather tedious process of ligaturing every vessel of appreciable size rising from the pancreatico-duodenal artery. We say this because in large animals of about 12 to 16 kilos hemorrhage may cause considerable delay.

As a dressing we have found the following preparation useful:

Gum mastic	220 grams
Styrax prep	135 grams
Benzol rectificatus	340 cc.
Oil of winter green	5.5 cc.

The animals were weighed before, and daily after the operation; they were fed on milk, meat and rice daily. They usually became markedly diabetic with a high blood and urine sugar content by the third day, and were usually used for experiment five to seven days after operation.

The isolated heart lung: This was carried out in the usual manner, care being taken to maintain a constant temperature, blood pressure and venous pressure; these were 37°C., 85 to 95 mm. Hg, and 15 cm. of water respectively. The output of the heart was carefully adjusted to the size and activity of the heart and maintained as nearly as possible at the figure obtaining during the first fifteen minutes of the experiment.

Anesthetic: Amytal was used as an anesthetic. Normal dogs received intraperitoneally 50 mgm. per kilo; diabetic dogs, which are very sensitive to this drug, received 15 to 25 mgm. per kilo. Changes in blood sugar during the first half-hour following amytal administration were not of the

large order indicated by certain workers (1, 2 and 3). This was undoubtedly due to the small dose given.

I. THE GLYCOGEN CONTENT OF NORMAL AND DIABETIC HEART MUSCLE. A series of estimations was made of the glycogen content of the right and left ventricles in whole animals and of hearts which had been isolated from two to four hours.

From table 1 it will be seen that the normal whole heart has a better glycogen supply than the heart which has been working cut off from the sugar stores of the body. The average for the whole heart being in the first case 0.590 gram per cent, in the latter 0.532 gram per cent. In the

TABLE 2

Effect of insulin on the glycogen content of normal and diabetic hearts

	WITHOUT INSULIN			WITH INSULIN		
	R. V.	L. V.	Number of experiments	R. V.	L. V.	Number of experiments
Normal						
Heart from whole animal	0.611	0.573	7	0.668	0.736	4
Heart-lung preparation not fed with sugar	0.563	0.502	5	0.689	0.729	5
Heart-lung preparation fed with sugar	0.931	0.762	2	1.206	1.143	2
Diabetic						
Heart from whole animal	0.866	0.852	3			
Heart-lung preparation with normal blood	0.753	0.723	5	0.439	0.363	2
Heart-lung preparation with normal blood and sugar				0.539	0.541	2
Heart-lung preparation with diabetic blood	0.401	0.265	3	0.357	0.289	4

diabetic the same thing obtains but at a higher level, the whole heart having 0.859 gram per cent, the isolated heart 0.741 gram per cent. It is also seen from table 2 that the addition of sugar to the perfusing blood causes the normal heart to synthesise glycogen.

II. THE EFFECT OF INSULIN ON THE GLYCOGEN CONTENT OF THE HEART. *Normal heart:* The action of insulin upon sugar storage depends upon whether or not the heart is isolated. The results given in table 2 show that the addition of insulin increases the power of the heart to store sugar as glycogen, and the extent to which it does so depends upon the richness of the sugar supply; the increase without sugar being 33.2 per cent, with sugar, 38.8 per cent. When the experimental heart has no sugar

added to the perfusing blood, the question as to whether or not there will be an increase in glycogen, depends upon the duration of the experiment as well as upon the initial percentage of sugar in the circulating blood. These results are in contradistinction to those of Hepburn and Latchford (4), who state that the glycogen content of perfused rabbit's hearts remained unchanged upon the addition of insulin. Their experiments were carried out however on excised rabbits' hearts perfused with Locke's solution. The results given here are from the isolated heart from heart-lung experiments of two hours' duration in which heart and lungs are perfused with mammalian blood. Results of experiments of longer duration and with a low initial blood sugar percentage, or where a marked hypoglycemia has been produced by previous insulin injections, are not given or discussed here.

The diabetic heart: The action of added insulin on the isolated diabetic heart is invariably to reduce glycogen storage, the fall under insulin from the high figure for glycogen of the diabetic heart being quite definite. Only in those cases where the blood sugar is maintained well above normal do we obtain any figures for glycogen approaching those for the normal heart. When the diabetic heart is perfused with diabetic blood and insulin added at the rate of 10 units hourly, there is no evidence of glycogen storage, and that despite a high blood sugar percentage. This is a result which to our surprise we have repeatedly found.

III. The utilisation of sugar in the normal and diabetic heart. The question of sugar utilisation has been the subject of much investigation. Locke and Rosenheim (5) were among the first to become engaged in this problem. Later MacLean and Smedley (6), Mansfeld (7), and Starling with his co-workers took up the question with regard both to the normal and diabetic organism. Utilisation figures for the normal excised perfused heart have varied from 0.5 to 7 mgm. per gram of heart muscle per hour. Knowlton and Starling (8) in 1912 found a marked difference between the sugar consumption of isolated normal and diabetic hearts. MacLean and Smedley (6) perfusing the hearts of diabetic dogs and cats with Ringer's solution concluded that the diabetic heart was unable to utilise sugar unless pancreatic extract were added to the perfusing fluid. In 1913 Patterson and Starling (9) came to the conclusion that there was little difference, if any, between the power of the normal and the diabetic heart to use sugar and, as a result of their work, they were inclined to abandon the view that the essential, or at any rate, the primary factor in diabetes is the absence of the power on the part of the tissues to consume sugar. Hepburn and Latchford (4) by experiments upon the excised normal rabbit's heart perfused with Locke's solution, showed that the addition of insulin to the perfusion fluid raised the sugar consumption from an average of 0.87 mgm. to an average of 1.90 mgm. per gram of heart muscle per hour.

The work of Burn and Dale (10) upon the isolated mammalian heart also demonstrated the fact that insulin increased the rate of removal of dextrose from the perfusing fluid. These workers also showed that if the blood sugar be prevented from falling by the infusion of sugar then insulin markedly increased the rate of disappearance of sugar. They also stated that isolated hearts from cats rendered diabetic by pancreatectomy gave results in all respects similar to those from normal animals, and they concluded that there can be no significant store of insulin in the muscles of normal animals. We are unable to corroborate these findings concerning the diabetic heart. A lack of uniformity in the results of perfusion experiments has been due to the varied methods employed. The isolated heart lung preparation has certain technical disabilities and unless careful check be kept upon extraneous factors responsible for loss of blood sugar, uniformity can not be expected. A preparation, which will last at least three hours with the minimal amount of lung edema in that time, can be secured by strict attention to the degree of inflation of the lungs, the arterial blood pressure, the venous inflow and the temperature.

A fact which has been overlooked in dealing with the question of sugar utilisation by the diabetic heart is that, in order to determine whether or not a purely diabetic heart will use sugar for energy or store it as glycogen, it is essential to perfuse such a heart with markedly diabetic blood. To perfuse with normal blood is merely to give the heart at the commencement of the experiment its normal amount of circulating insulin with the result that, for a certain period, restoration of lost power is effected.

The method for the calculation of sugar used is of importance (see protocol). Sugar is lost principally through the loss of blood arising from edema of the lungs, and by leakage occasioned by the use of defibrinated blood, and unless this loss be accounted for accurate results can not be obtained.

EXPERIMENTAL RESULTS. In discussing the results given in tables 3 and 4 we have to consider the utilisation figures in relation to glycogen content and free muscle sugar and the action of insulin thereon under 1, blood sugar of a normal content, and 2, blood sugar which has been increased and maintained well above normal.

1. Utilisation in normal hearts without insulin. The data in table 3A show that, in isolated heart lung experiments of two to three hours' duration in which normal blood is used and no sugar added, the dog's heart utilises sugar at the average rate of 2.24 mgm. per gram of heart muscle per hour. The degree of utilisation of blood sugar depends upon a balance between circulating sugar and stored glycogen. The high sugar content of the blood used for perfusion is due to ether anesthetisation of the bled animal. It would appear from a consideration of the glycogen content of the heart of experimental animals, that while blood sugar is utilised

the heart glycogen is also drawn upon for energy. The free muscle sugar under these experimental conditions averaging as it does 20 mgm. per cent is probably slightly below the normal for the whole animal.

The effect of the addition of sugar to the blood is seen in table 3B, where the average initial blood sugar content was 0.393 gram per cent and the final at the end of three hours 0.271 gram per cent. The utilisation shows a threefold increase over the normally perfused heart and a marked increase in the deposition of glycogen. It is clear that with a sugar supply above normal the heart increases its sugar utilisation, the degree of utilisation depending upon the concentration of blood sugar and the increase in the ability of the heart to utilise the energy liberated by increased oxidation to synthesise part of the sugar into glycogen. The free muscle sugar figures of 84 and 68 mgm. per cent are probably due to the high blood sugar content.

Perfusion of the normal heart with diabetic blood (table 3C) results in a utilisation figure of 4.61 mgm. per gram of heart muscle per hour. It may be that this high utilisation is due to the high blood sugar concentration and to the presence of a small amount of insulin in the heart muscle; that insulin is present only in small amounts, amounts sufficient for utilisation over the period of the experiment but not sufficient for synthesis, may be inferred from the fact that under these conditions the heart suffers a diminution of its glycogen store. The dogs from which blood was taken were four days diabetic and it is probable that this blood was not insulin free. The free muscle sugar has been increased, the glycogen reduced.

2. Utilisation in normal hearts with insulin. In table 3D the effect of the addition of insulin to normal blood perfusing normal hearts is shown. It is to be noted that sugar has been made to disappear rapidly from the blood, a fall of from 0.209 to 0.077 gram per cent occurring in two hours. The utilisation has been increased to about three times the normal, namely, 6.22 mgm. per gram of muscle per hour, a result which is not in agreement with the statement by Plattner (11) that the heart-lung preparation does not increase the rate of disappearance of blood sugar upon the addition of insulin so long as the heart rate is not increased. As can be seen from our protocols, in none of our experiments was there any variation in the rate of the isolated heart upon insulin administration. Here also the glycogen content has been increased by about 200 mgm. as is seen from a comparison of these results with those in table 2, and table 3A. These results are comparable to those obtained by Burn and Dale (11) on the excised rabbits' hearts perfused with Locke's solution, in that they demonstrate an increased utilisation of blood sugar and an increased deposition of glycogen in the heart muscle. The addition of insulin to blood with a high sugar content produces a very marked increase in utilisation; and not only is there an increased utilisation amounting to 11.76 mgm. but a great in-

crease in the amount of glycogen and in the free muscle sugar. Here then is the combined effect of insulin and sugar feeding and it is in accordance

TABLE 3

Sugar utilisation of normal hearts in milligrams per gram of heart muscle per hour showing relation to the glycogen and free sugar of the heart, with and without insulin

	BLOOD SUGAR PER CENT		GLYCOGEN		MUSCLE SUGAR		UTILIZATION PER GRAM PER HOUR	PERIOD
	Start	End	R	L	R	L		
Without insulin								
	gram	*gram*	*gram*	*gram*	*mgm.*	*mgm.*	*mgm.*	*hours*
A. Normal heart with normal blood: No sugar added	0.214	0.122	0.584	0.533	28	22	2.72	3½
	0.171	0.137	0.551	0.494	21	18	2.62	2
	0.166	0.109	0.669	0.470			1.54	2
	0.187	0.101	0.421	0.646	17	19	2.10	3½
Average	0.184	0.117	0.555	0.535	22	20	2.24	2¾
B. Normal heart with normal blood; sugar added to maintain per cent above normal	0.342	0.226	0.793	0.640			4.04	2½
	0.453	0.328	1.070	0.883	84	68	8.70	3
	0.384	0.261	0.840	0.730			7.08	3
Average	0.393	0.271	0.901	0.751	84	68	6.61	3
C. Normal heart with diabetic blood	0.447	0.381	0.401	0.380	41	35	4.66	2½
	0.292	0.131	0.354	0.266			4.56	3
Average	0.369	0.256	0.378	0.323	41	35	4.61	2¾
With insulin								
D. Normal heart with normal blood: 10 units insulin half hourly. No sugar added	0.196	0.051	0.827	0.804	10.7	10.6	7.02	2
	0.222	0.104	0.657	0.634	17.0	13.0	5.42	2
Average	0.209	0.077	0.742	0.719	14	12.0	6.22	2
E. Normal heart with normal blood: 10 units insulin with sugar added	0.580	0.427	1.152	1.336	85	105	10.60	3
	0.560	0.332	1.260	0.950	95	111	14.53	3
	0.457	0.311					10.15	3
Average	0.532	0.357	1.206	1.143	90	108	11.76	3

with the suggestion of Bissinger, Lesser and Zipf (12), that when insulin is injected in the presence of an excess of blood sugar, part of the sugar will be burned and part synthesised as glycogen.

3. Utilisation in diabetic hearts without insulin. The perfusion of a diabetic heart with normal blood results in a utilisation figure comparable to that obtained in the normal heart perfused with diabetic blood, namely, 4.15 mgm. per gram of heart muscle per hour. In table 4A it will be seen that in two experiments of three hours' duration in which the animals were five days diabetic the utilisation is enhanced, the glycogen remains at a figure which is that obtained in the diabetic heart which would indicate that no glycogen has been broken down and that the energy of the heart has been obtained by the oxidation of blood sugar. It must be noted however that the initial blood sugar content is about twice the normal while the final percentage is not below the normal. From a comparison of these results with those for the normal heart perfused with diabetic blood it would appear that insulin in normal blood, where it is under strictly physiological conditions and in physiological amount, is of much more value in maintaining the glycogen content of the heart at or about normal, than is the insulin contained in the heart muscle.

The diabetic heart perfused with diabetic blood: Much has been written about the power of the diabetic heart to use sugar and the discrepancies in results can only be attributed to the fact that the hearts were either not completely diabetic or that the blood with which they were perfused was not insulin free. Given the minimal amount of insulin either in cardiac muscle or in the circulating blood that is possible for the maintenance of life, then the ability of the heart to utilise circulating or stored dextrose will be minimal. Table 4 B shows that five days after pancreatectomy dogs still have in circulation an amount of insulin sufficient to effect a certain degree of sugar utilisation but apparently not sufficient to maintain the glycogen storage within their hearts.

As far as their blood sugar content is concerned these dogs are all markedly and almost equally diabetic but blood sugar percentages afford no indication of the extent to which insulin may be present in the blood and tissues.

With an experimental dog two days diabetic and a bleeder four days diabetic there is still a definite sugar utilisation and no marked change in glycogen content but with increasing duration of the diabetic condition we find that despite high blood sugar percentages there is a fall both in sugar utilisation and in the glycogen content of the heart. A consideration of the data in table 4B will show that with progressive diabetes the ability to use sugar becomes steadily diminished and the power to synthesise glycogen is entirely lost. These figures represent a severe if not extreme diabetic condition and point to a rapid elimination of insulin from the heart and circulating blood. The free sugar is high as a mechanical result of a high blood sugar. In one experiment upon dogs diabetic for eight days we obtained after correction for the *normal* sugar utilisation of the lung, a negative figure, -0.09 mgm. for the utilisation of the heart.

It is clear that the part played by the lungs in this condition requires investigation.

TABLE 4

Sugar utilisation of diabetic hearts in milligrams per gram of heart muscle per hour showing relation to the glycogen and free muscle sugar of the heart, with and without insulin

	BLOOD SUGAR PER CENT		GLYCOGEN		MUSCLE SUGAR		UTILIZATION	DURATION OF EXPERIMENT	DURATION OF DIABETES	
	Start	End	R	L	R	L			Experimental dog	Bled dog
					Without insulin					
	gram	*gram*	*gram*	*gram*	*mgm.*	*mgm.*	*mgm.*	*hours*	*days*	*days*
A. Diabetic heart with normal blood	0.192	0.131	0 875	0 860	35	29	4 56	3	5	
	0 272	0 128	0 671	0 615	28	29	3 74	3	5	
Average	0 232	0.129	0 773	0.737	32	29	4.15	3	5	
B. Diabetic heart, diabetic blood	(1) 0.418	0.239	0 604		89	81	2 58	3½	2	4
	(2) 0 342	0 282	0 401	0 307	78	80	1.15	2	5	5
	(3) 0.403	0 342	0 201	0 223	74	69	0 59	4	9	8
	(4) 0 369	0 291	0 495	0 241	78	80	0 09	4	8	8
(1) Blood sugar: Experimental dog = 0.392 gram per cent; bled dog = 0.480 gram per cent.										
(2) Blood sugar: Experimental dog = 0.574 gram per cent; bled dog = 0.354 gram per cent.										
(3) Blood sugar: Experimental dog = 0.382 gram per cent; bled dog = 0.442 gram per cent.										
(4) Blood sugar: Experimental dog = 0.411 gram per cent; bled dog = 0.403 gram per cent.										
					With insulin					
C. Diabetic heart with normal blood. Insulin 10 units half-hourly	(1) 0 254	0.128					5.11	2½	5	
	(2) 0.220	0 101	0 482	0 382	47	30	4 68	3	5	
	(3) 0 194	0 097	0 397	0 344	42	34	4 89	3	4	
Average	0 223	0 108	0.439	0 363	44	32	4 89	3	5	
Blood sugar (1) Experimental dog = 0.358 gram per cent.										
(2) Experimental dog = 0.377 gram per cent.										
(3) Experimental dog = — gram per cent.										
D. Diabetic heart with normal blood: sugar added. Insulin 10 units half-hourly	(1) 0.497	0 442	0 587	0 507		160	8 51	3	4	
	(2) 0 452	0 389	0 491	0 575		120	7 10	2½	5	
Average	0 474	0 415	0 539	0 541		140	7 80	3	4½	
Blood sugar (1) Experimental dog = 0.447 gram per cent.										
(2) Experimental dog = 0.563 gram per cent.										
E. Diabetic heart, diabetic blood. Insulin10 units half-hourly	(1) 0 477	0 230	0 192	0 164	48	50	7 73	2½	6	6
	(2) 0 453	0 266	0 295	0 221	53	49	7 50	3	5	5
Average	0 465	0 248	0 243	0 192	50	49	7 61	3	5½	5½
Blood sugar (1) Experimental dog = 0.562 gram per cent; bled dog = 0.412 gram per cent.										
(2) Experimental dog = 0.403 gram per cent; bled dog = 0.487 gram per cent.										

4. Utilisation in diabetic hearts with insulin. The diabetic heart perfused with normal blood to which insulin has been added has its sugar utili-

sation increased to an average of 4.89 mgm. per gram of heart muscle per hour, an increase which is comparable to that obtained with the diabetic heart perfused with normal blood.

Insulin has occasioned not only an increased disappearance of sugar from the blood, but it has caused a definite fall in the glycogen content of the heart. This increased degradation of glycogen may be responsible for the higher level of free muscle sugar within the heart. In those diabetic hearts where insulin in physiological dosage increases sugar utilisation without degradation or with a synthesis of glycogen the free muscle sugar tends towards the normal figure of 25 mgm. per cent (table 4A and C). On the other hand added insulin in the presence of a high blood sugar level still does not effect any increased synthesis of glycogen in the diabetic heart despite the increase in sugar utilisation (table 4D).

It would seem from these results that insulin in excess causes a breakdown of glycogen which can only be held in check by the continual addition of dextrose to normal blood.

It has been shown that the diabetic heart perfused with diabetic blood fails in a large degree to utilise sugar. That insulin restores this lost power is clearly demonstrated in table 4E where the addition of 10 units half hourly gives an average utilisation figure of 7.61 mgm. per gram of heart muscle per hour. Here where there is little or no insulin circulating under physiological conditions, the addition of excess of insulin leads to a breakdown of heart glycogen, due probably to an increase in the metabolic activity of the heart.

The high free muscle sugar content of the diabetic heart, due to a high blood sugar percentage, is considerably reduced. It is quite probable that this effect of added insulin is not a normal result and that to arrive at some clearer idea as to the action of insulin upon diabetic heart glycogen, it should be given in its physiological habitat, namely, normal blood.

Discussion. In a discussion of the carbohydrate metabolism of the normal and diabetic heart one is at once faced with the peculiarity that in contradistinction to the normal heart, the diabetic heart stores glycogen. This cannot be taken in support of the idea that insulin is not necessary for the synthesis of muscle glycogen because the diabetic organism is not necessarily insulin free. With the progress of insulin elimination there comes a point when the heart, with a minimal amount of insulin, unable to use sugar and unable to synthesise glycogen, has recourse to its sugar stores, with the result that we find a diminution of heart glycogen (tables 2 and 4B). In considering the action of insulin upon sugar utilisation and storage one must have regard to the balance between three variables, namely, blood sugar, muscle sugar and muscle glycogen. It is also clear that in any discussion of the subject as far as it is dependent upon results secured from perfused isolated heart lung preparations, the duration of the experiments and of the diabetic condition must be taken into account.

In tables 3 and 4, figures for free muscle sugar are given. The heart of the normal whole animal contains approximately 25 mgm. per cent of free sugar and the isolated heart a little less, probably due to the steadily diminishing concentration of blood sugar. Feeding the normal heart with sugar raises the muscle sugar. It is clear that the percentage of free sugar is dependent upon the concentration of blood sugar. Raising or lowering the blood sugar, as in diabetes or by insulin administration, will immediately cause concurrent and similar changes in the concentration of free muscle sugar. It is seen that in diabetes the muscle sugar averages about 80 mgm. per cent and that the addition of insulin, restoring the normal concentration of blood sugar, tends to bring the muscle sugar back to normal.

The action of insulin in the normal heart depends upon the quantitative relation of insulin to available sugar (12). The isolated normal heart contains, in approximate figures, 0.550 gram per cent of glycogen, the blood sugar averages 0.110 gram per cent, muscle sugar 21 mgm. per cent and the utilisation, when no sugar is added to the perfusing blood, averages 2.24 mgm. per gram of heart muscle per hour (table 3A). When sugar is added and a *high blood sugar percentage is maintained* all of these figures are increased (table 3B). The addition of insulin in presence of sugar leads to a further increase, viz., glycogen about 1.200 gram, muscle sugar 100 mgm., utilisation 11.76 mgm. (table 3E). It is clear from such results that insulin increases sugar utilisation and glycogen synthesis, and that synthesis in the isolated normal heart demands a high blood sugar to meet the injected insulin. This bears out on the isolated heart what has been demonstrated on the eviscerated preparation by Dale and his co-workers (13). If however the blood sugar be not maintained the question as to whether glycogen will be stored or broken down will depend upon the rate of occurrence of hypoglycemia, its degree and duration.

The figures for utilisation give no clue to the total oxidation of sugar by the heart and the fact that under such conditions as perfusion of a normal heart with diabetic blood, there is a breakdown in glycogen has at present no adequate explanation, although it may be regarded as evidence that the heart muscle contains only a small amount of insulin.

In the diabetic heart the interpretation of results is more difficult. In the heart of the diabetic animal, with its comparative lack of insulin, a high glycogen content is associated with a high muscle and a high blood sugar. As a result of perfusion with normal blood, which means merely restoring the normal quota of insulin, the general effect is restoration to normal function. The figure for sugar utilisation returns to that which is approximately the normal for a heart supplied with a *maintained normal amount* of circulating sugar (2 to 4 mgm. per gram per hour), table 4A. That a rapidly progressive diabetes results in an elimination of circulating

insulin, a consequent diminution if not cessation of utilisation of blood sugar, and a marked loss in heart glycogen is we believe a correct deduction from the experimental evidence given in table 4B.

The high muscle sugar with the increase in glycogen, which obtains in diabetes, results from the increased blood sugar concentration and the diminished ability of the muscle to metabolise sugar. Insulin by restoring this function immediately brings about a lowering of the muscle sugar towards its normal level, and a reduction in blood sugar. In virtue of a high blood sugar concentration obtaining in the diabetic an enhanced utilisation is the immediate result of perfusion with normal blood containing a *physiological* amount of insulin. By the addition of insulin sugar utilisation is further increased, but such an increase only obtains as long as the blood sugar is above normal, for the ultimate result of large doses of insulin is to reduce the percentage of both blood and free muscle sugar and, if such reduction be allowed to progress, the inevitable result will be the production of hypoglycemia, a depletion of glycogen storage and finally a decrease in the utilisation of sugar.

A result which at first sight appears wholly unexpected, is the fall in the glycogen content of the diabetic heart under the action of insulin. If in the normal heart insulin brings about the synthesis of glycogen in the presence of a maintained normal sugar level (table 3D and E), why should there be an absence of glycogen synthesis in the diabetic heart, which has had its power of sugar consumption restored by added insulin (table 4C, D, E)? The fall in glycogen is certainly not due to a fall in blood sugar, neither can it be supposed that the action of insulin in stimulating sugar consumption is totally different from, and uncorrelated with its action on glycogen. It is true that *the optimum insulin concentration necessary for glycogen synthesis has not been determined, neither has it been disproved that excessive doses of insulin cause a breakdown of the glycogen molecule.* The question as to whether the action of insulin in stimulating sugar consumption in the diabetic state will be associated with glycogen synthesis or glycogen degradation depends upon a consideration of the balance maintained between the blood sugar content, the concentration of free sugar in the muscle, the degree of glycogen storage and the rate of sugar consumption by the heart muscle.

SUMMARY

1. Blood sugar, muscle sugar and glycogen of the heart have been estimated in the case of normal and diabetic dogs, both in the whole animal and under isolated heart lung conditions, and the effect of insulin thereon has also been studied.

2. The disabilities of the isolated heart lung preparation have been pointed out and means have been indicated for overcoming these in order that consistent results may be obtained.

3. The utilisation of sugar by isolated normal and diabetic hearts has been studied and the effect thereon of normal and high blood sugar concentrations and of insulin administration has been determined.

It has been established both for the normal and diabetic heart that sugar utilisation is increased by the addition of insulin and also by maintaining the blood sugar concentration at a high level.

The facts, that the diabetic heart perfused with diabetic blood will not utilise blood sugar and that this lost power is restored by perfusion with normal blood or by the addition of insulin, have been demonstrated.

4. The concentration of muscle sugar has been found to be dependent upon the concentration of sugar in the blood. The effect of insulin upon muscle sugar is the outcome of its action upon blood sugar.

5. The facts bearing upon glycogen synthesis have been studied. It has been show that, whether or not under experimental conditions in the normal heart glycogen will be synthesised or broken down, depends upon insulin dosage and blood sugar concentration.

In the diabetic heart insulin administered in physiological doses maintains glycogen synthesis, and brings the glycogen and muscle sugar content towards the normal.

6. The factors controlling the balance between blood sugar, sugar utilisation and sugar synthesis, as shown by experimental results, have been discussed.

Protocols of experiments

Dog 64.
Date of admission = 18/3/28. Weight = 7.50 kilos
Date of depancreatectomy = Weight =
Date of experiment = 22/3/28. Weight = 7.25 kilos
Anesthetic = Amytal. Blood sugar =
A. *Experimental procedures.*
Heart isolated 10:0 a.m.
Sample of blood taken from reservoir for blood sugar estimation.

TIME	TEMPERATURE	HEART RATE PER MINUTE	RHYTHM	A. B. P.	V. B. P.	OUTPUT CC. PER MINUTE	VOLUME BLOOD IN RESERVOIR
	°C.			*mm. Hg*	*cm. Aq.*		
10:5	36	144	Normal	85	15.5	420	540
10:35	37	152	Normal	85	15.0	420	525
11:5	37	152	Normal	85	15.0	450	510
11:35	37	152	Normal	85	14.5	450	495
12:5	37	152	Normal	85	14.0	450	470
12:35	37	152	Normal	85	13.5	420	445
1:5	37	152	Normal	85	12.5	360	400
1:35	37	152	Normal	90	12.5	300	300

B. *Method of correcting for loss of blood, and loss of sugar in calculating sugar utilisation.*

The first period only is given as an example.

Time.

10:5 Total volume of blood in schema, heart and lungs = 540 + 350 = 890 cc.
Percentage of sugar in the blood = 0.214
Therefore total amount of sugar present = 0.214 × 8.9 = 1.905 gram

10:35 Total volume of blood in schema, heart and lungs = 525 + 350 = 875 cc.
Percentage of sugar in the blood = 0.196
Therefore total amount of sugar present = 0.196 × 8.75 = 1.715 gram
Volume of blood lost during the interval = 15 cc.

$$\text{Percentage of sugar in the blood lost} = \frac{0.214 + 0.196}{2} = 0.205 \text{ gram}$$

Therefore the amount of sugar lost = 0.205 × 0.15 = 0.031 gram
Therefore amount of sugar available for utilisation = 1.905 − 0.031 = 1.874 gram
Hence sugar utilised in the first half-hour = 1.874 − 1.715 = 0.159 gram

C. *Method for calculation of utilisation per gram weight of heart per hour.*

1 PERIOD	2 TOTAL SUGAR PRESENT	3 PERCENTAGE SUGAR	4 SUGAR LOST	5 TOTAL SUGAR AVAILABLE	6 TOTAL SUGAR UTILISED
hours	*gram*	*gram*	*gram*	*gram*	*gram*
0	1.905	0.214			
			0.031	1.874	0.159
½	1.715	0.189			
			0.029	1.686	0.061
1	1.625	0.189			
			0.025	1.600	0.333
1½	1.268	0.150			
			0.038	1.230	0.033
2	1.263	0.154			
			0.038	1.225	0.032
2½	1.193	0.150			
			0.063	1.130	0.147
3	0.983	0.131			
			0.127	0.856	0.063
3½	0.793	0.122			

Weight of heart = 53 grams
Total sugar utilised = + 0.762 gram
Sugar utilised by lungs per gram of heart muscle per hour − 1.40 mgm. (Starling)
∴ sugar utilised per gram of heart muscle per hour = 2.72 mgm.

Results:

Blood sugar = 0.214 to 0.122 gram per cent
Muscle sugar = R.V. = 28 mgm. L.V. = 22 mgm.
Glycogen = R.V. = 0.584 gram L.V. = 0.533 gram
Utilisation = 2.72 mgm. per gram per hour

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THE EFFECT OF REPETITIVE STIMULATION UPON THE DURATION OF THE ELECTRICAL RESPONSE OF VENTRICULAR MUSCLE

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Previous stimulation has long been known to produce definite effects upon conduction rate and contractility of heart muscle, especially as evidenced in treppe of contractility. Ashman (1925) has recently contributed to certain aspects of our knowledge with respect to treppe and fatigue in conduction between auricles and ventricle and between the right and left auricles. The presence of treppe and subsequent fatigue effects as revealed by electrical records is to be expected if there is a strict parallelism between electrical and mechanical responses of heart muscle. It was to investigate the nature of these effects that the series of experiments reported here was undertaken.

Ventricular strips were prepared in most cases from hearts of P. concinna or C. marginata. A few experiments done with strips from other species of turtles showed no qualitative difference in the results produced. Repetitive stimuli were thrown into the strip at such rates that the strip usually responded to every stimulus. Stimuli were applied near one end of the strip and leads were taken from near the other end and either at the stimulated region or at the frenum. Stimulation strength was selected at 5 per cent to 10 per cent above threshold at the beginning of the experiment. In course of the experiments three devices were employed at one time or another for stimulating. The first instrument used was a Harvard induction coil activated by two dry cells, the primary current being made and broken by a slowly rotating interrupter, break shock only being used for stimulation. The make shocks were short-circuited through a second contact on the interrupter. The second device was suggested by Dr. C. J. Campbell and consisted of a three element thermionic tube (201A type) used in an oscillator based on the Hartley feed-back circuit. The transformer was an inexpensive 3-1 audio transformer which gave single, sharply peaked impulses over a considerable range of frequencies. Several transformers which were tried lacked the proper damping powers of the one finally used with the result that with them, each discharge was made up of a series of decrementing oscillations.

This complicating oscillation has been considered by Campbell (1929) in a recent paper. As the modified instrument which he describes appears to be more readily controlled and calibrated, further discussion of the stimulator as we used it seems unnecessary. The third device used was based on the instrument described by Scheminsky (1928). In this instrument a Cooper-Hewitt, ½ watt neon glow lamp was used to produce rhythmic partial discharge of a condenser charged through the plate circuit of a 199A type tube used as a variable resistance. The laboratory 220 volt d.c. circuit was used on the plate circuit. This device gave easily controlled frequencies and stimulating strengths over a fairly wide range.

A capillary electrometer with a one panel amplifier served as recording instrument. Photographic records were taken at intervals during periods of stimulation. From these records measurements were made of the conduction time through part or all the strip. The results so obtained were always qualitatively comparable, but conduction time measurements which included the apex often showed a somewhat longer conduction time proportionately than other records. This was apparently due to increased delay at the apical region with progressive fatigue. The duration of the sustained electrical response was measured by the interval between the beginning of the R-wave and the beginning, or the peak of the T-wave. The times as measured by these two methods were always nearly parallel except as delayed conduction lengthened the time from T-start to T-peak. With excessive fatigue, the T-wave becomes much flattened and broadened, but the curves shown here were not obtained from preparations which had been fatigued to that extent.

As will be seen from figure 1 or figure 2, the outstanding changes of the electrogram produced by continued stimulation are the slowed conduction and the shortened R-T interval. The rate at which these changes occur is largely dependent upon the rate of stimulation of the strip. Rapid stimulation with intervals of 2 to 3 seconds causes a much steeper slope in the early part of the curves and a somewhat greater change than is produced by slower stimulation, with intervals of 5 to 6 seconds. The decrease in the R-T interval due to this fatigue condition passes off to some degree during longer rest periods. This is demonstrated by the curve of figure 2D. This curve was prepared from a series of records obtained by adjusting the interrupter to give stimuli at intervals of about 2.5 seconds but with every third shock short-circuited by means of a hand switch. The resultant alternately short and long cycles of the strip were then plotted as shown. Using the zero on the abscissa as indicating the time of the first shock applied to the strip after a 15 minute rest period, continuous time in seconds is plotted along the abscissa. The duration of the R-T interval is plotted along the ordinate, curve 1 representing the lengths of the interval from the start of R to the peak of T, and curve 2 representing the interval

from the start of R to the start of T. During the longer rest periods, the recovery of the heart is more complete and the R-T interval is longer than is the case after the shorter rest periods with less complete recovery.

Figure 2A for example shows the effect of continued stimulation over a period of nearly 200 seconds. For such experiments there results a continued shortening of the R-T interval and a lengthening of conduction time, except for the treppe effects. Treppe is frequently but not invariably observed, both for conduction and for duration of the sustained electrical

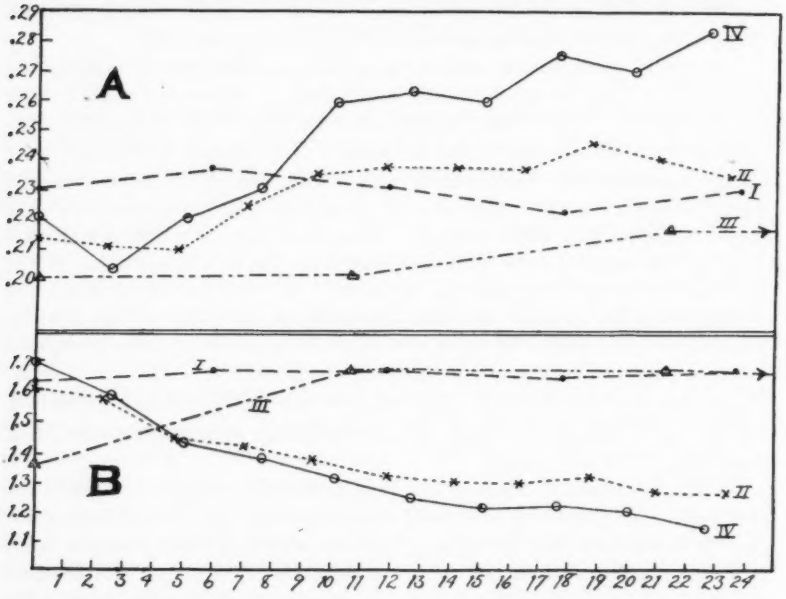

Fig. 1. Conduction time (A) and intervals from start of R-wave to peak of T-wave (B) plotted as ordinates against continuous time as abscissae. After the responses plotted as curves I, the strip rested without stimulation for 7 minutes. Responses plotted as curves II were then elicited. After a rest of 12 minutes there followed responses for curves III, then a rest of 10 minutes and the responses for curves IV.

change. In the case of conduction (fig. 1A, curves II and IV) treppe is seen as a quickening of the conduction time. Treppe of the duration of the electrical reaction (fig. 2, A, B and C) is seen as a lengthening of this period for a few beats, after which the gradual and slightly irregular falling off occurs. The effect of gradual fatigue in the excised heart in a series of several such stimulation periods is also to be seen from either figures 1 or 2. The conduction time becomes increasingly longer and the R-T interval curve shows a sharper falling off at the start and a somewhat lower final value.

The results presented above are entirely compatible with many records which we have obtained showing a shortened systolic time for premature

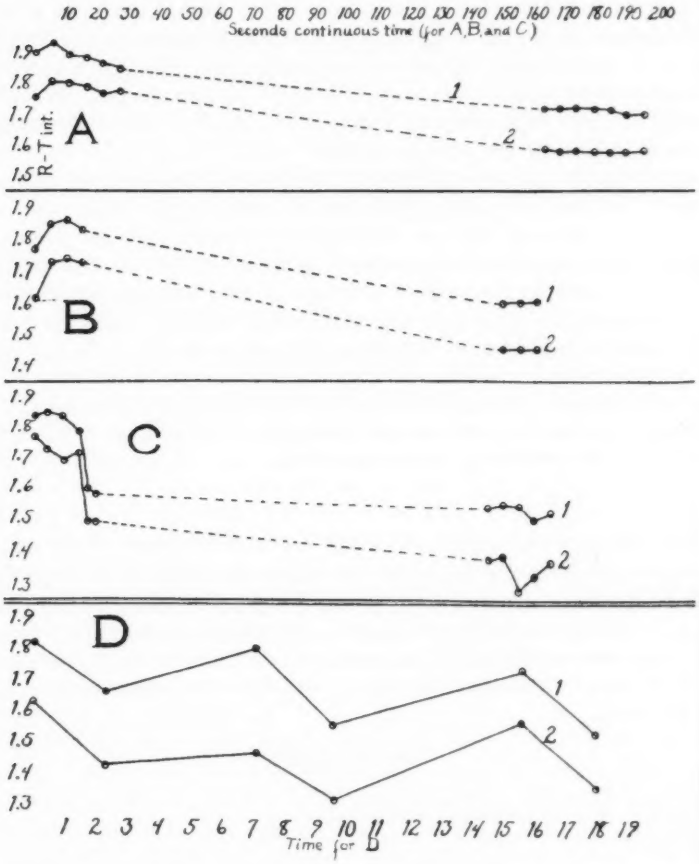

Fig. 2. Intervals from start of R-wave to start, *1*, and peak, *2*, of T-wave respectively plotted as ordinates against continuous time as abscissa. Note that abscissa intervals for D are not the same as those for A, B, and C.

Rest periods, between A and B, 16 minutes; between B and C, 13 minutes; D being plotted from responses obtained from same strip later in experiment after a 15 minute rest period.

contractions. Nor are they incompatible with some mechanical records taken during periods of continued stimulation and showing apparent lengthened systoles. For it is often difficult in such records to separate

the tendency of increased total systolic time, due to a somewhat shortened local systole but with a more than corresponding increase of the conduction time. In one of our experiments, for example, (expt. 55282) the curve of the interval from the start of the R-wave to the start of the T-wave could not be accurately plotted. A plot of the interval from the start of R to the peak of T gave, not a falling, but a rising curve. Measurement of the conduction time showed an increase of from 0.3 to 0.85 second. When the conduction time values were subtracted from the R-T interval values as measured the usual falling curve resulted.

Concerning the occurrence of treppe of electrical duration, we can name no set of "normal" conditions which will invariably give or fail to cause its appearance. Ashman (1925, p. 141) seems to correlate the appearance of a supernormal phase and treppe of A-V conductivity with warm weather (35° to 37°C.). Our best effects of treppe of R-T interval were recorded from preparations with room temperature of 25° to 27°C. and two experiments done at temperatures of 30° to 32°C. showed no R-T treppe whatever. Experiments done in the spring (April and May) of two years, showed R-T treppe in a higher percentage of preparations than did experiments done in the fall (October and November). At neither time of year was R-T treppe constantly present or absent.

SUMMARY

With repeated stimulation of turtle ventricular strips continued for several minutes, there is a gradual increase of the conduction time. The local systolic time, as measured by the R-T interval becomes shortened. Treppe of conduction and of the duration of the sustained electrical reaction may occur during the first few responses of such a series. During the period of treppe, there is quickening of conduction and a lengthening of the R-T interval.

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EFFECTS OF COLD AND OF VERATRIN UPON THE ACTION POTENTIAL OF VENTRICULAR MUSCLE

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Records were made by use of a one or two panel amplifier connected with a capillary electrometer or Braun tube (cathode ray oscillograph), respectively. The arrangements used were those outlined in a previous paper (Gilson, 1929). As was shown in that paper, the time elements of the strip electrogram which seemed significant were those 1, from the beginning to the crest of the rapid rise of the R-wave as a measure of the time of development of the local (relative) negativity at the proximal lead-off electrode; 2, from the beginning of rise to the beginning of the rapid fall of the R-wave as indicative of the time required for conduction of the impulse from the proximal to the distal electrode, and 3, from the beginning of the R-wave to the beginning of the T-wave as a measure of the duration of the "monophasic platau." The height of the R- and T-waves and the form of the T-wave must also be considered in a complete treatment of the electrogram. In the experiments reported here the changes in height of the waves in response to treatment were rarely great enough to be significant until very great depression was produced. At that time both R- and T-waves were depressed in height.

EFFECTS OF COLD. For each experiment, a ventricular strip was suspended in a glass and fiber box provided with binding posts on the outside so that, after the strip had been properly arranged, the box did not need to be opened during the course of an experiment. A normal diphasic electrogram was obtained and the closed box with the strip was then placed in the laboratory ice-box which had a temperature close to zero centigrade. About twenty minutes was required for the temperature within the moist box to be lowered to 10°C. At some time before this point was reached, the moist box was taken from the refrigerator, carried to the dark room, connections made to the recording and stimulating apparatus and records taken. As the temperature within the box rose slowly, it was usually considered justifiable to assume that the true temperature of the strip lay within one degree of that read from the thermometer placed in the box.

Figure 1A is a Braun tube record of the R-wave obtained from a strip at 22°C. Figure 1C and figure 1D were obtained from the same strip after it had been cooled to 12°C. and then allowed to warm to 16°C. Figure 1I and figure 1J are diagrams of the form of the entire electrograms obtained

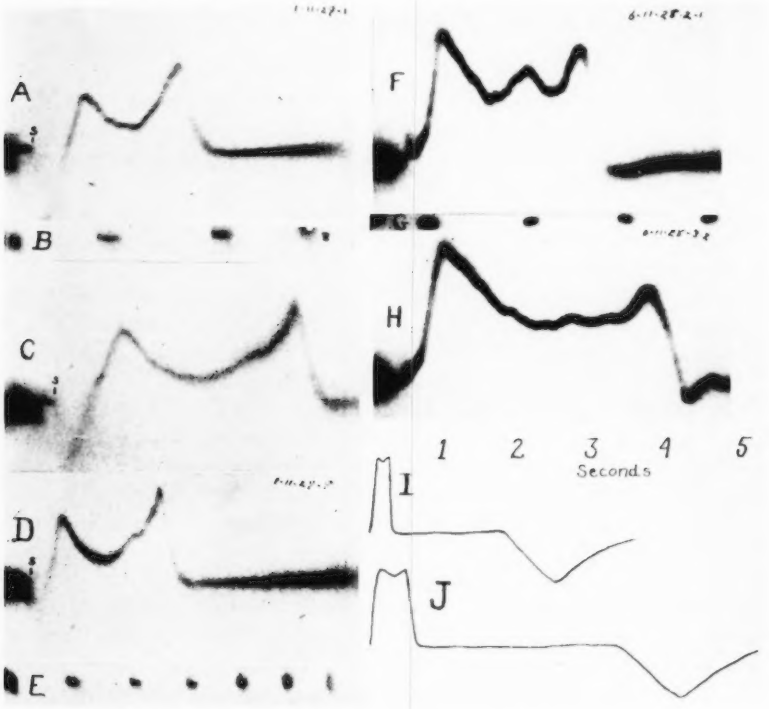

Fig. 1. A, C, D, F, and H. Cathode ray tube records from strip preparations, initial complexes only. A, temperature 22°C. B, time record for A and C, intervals of $\frac{1}{5}$ second. C and D, records from same strip as A but with temperature 12°C. E, time record for D, intervals of $\frac{1}{5}$ second. F, "normal." G, time record for F and H, intervals of $\frac{1}{5}$ second. H, record from same strip as F but after veratrin poisoning. I and J, diagrams of electrograms from strip at 22°C. and 16°C. respectively. Time as indicated.

from this strip at 22° and at 16° respectively. It will be seen that under the effect of the lowered temperature all of the processes involved in the production of the electrogram are slowed to nearly half the rate seen at 22°. The relative prolongation of the R-T interval is less than that of the other intervals involved. Figures 1A and 1C were taken with the same

rate of movement of the oscillograph spot. Figure 1D was taken with a rate of spot movement almost exactly one-half that of the other two records and, as it happened, the two rates were such that the fast record of figure 1A and the slower one of figure 1D may be almost exactly superimposed. Such precise similarity of change of the rising time of the R-wave and of the time of conduction does not always occur. The two may show changes independently and with apparently different temperature coefficients. In all cases, however, all the processes of the cardiac reaction are slowed by the lowered temperature and in somewhat similar, though usually not identical proportions.

Effects of veratrin. Veratrin sulphate, 0.1 per cent in Ringer's solution, was applied to ventricular strips by immersing the strip used in a given experiment in a small vessel containing the solution and without removing the strip from the wicks of the calomel eletrodes. The vessel containing the fluid was removed from under the strip during the actual taking of records. Records were also obtained from excised perfused ventricles.

The first effect of veratrin treatment is often seen in a slight and very brief shortening of the time of conduction. Occurring more regularly and persisting for a longer time is a shortened duration of the sustained diphasic and (by analysis) of the monophasic electrical response, a shortening which may be as much as 0.2 second. During this first stage, the threshold to stimulation by induction shocks is considerably lowered.

Following this temporary quickening of activities, there is a pronounced slowing of the conduction rate. In one case (6–11–28) this increase was from a normal of 0.4 second to a value of 1.3 second under veratrin effect. During this stage the rising time of the R-wave rarely shows significant changes, although the threshold begins to rise. The duration of the monophasic plateau does not increase at this time although the *peak* of the T-wave may fall later because of the lengthened conduction time. Later effects of veratrin usually cause a marked delay in the beginning of the T-wave. In nearly all the experiments done, the duration of the time to the beginning of the T-wave could be increased from a normal maximum of 1.8 seconds (usually less than this in spring turtles) to 2.5 or 3.0 seconds. The relatively great prolongation found by other workers came on still later or might never develop. It seems probable that at least part of the regular prolongation of apparent systole found under veratrin poisoning is due to the very much lengthened conduction times which are to be found. In many of our preparations relaxation commenced at the proximal end of a strip almost as soon as contraction began at the distal end of the strip.

As the poisoning becomes more severe, there is a lengthening of the time of the rising phase of the R-wave. This lengthening occurs at first without any decrease in the height of the R-wave. Still further depression

results in a still greater slowing of the R-wave rise and a fall in the height of the wave. The T-wave meanwhile becomes broadened and flattened.

An interesting complication of the veratrin effects is the fact that in many records there is definite evidence that at certain points along a strip (or in a perfused ventricle) there are regions which are peculiarly susceptible to block. Impulses traveling at a slowed but fairly constant rate will be delayed at such regions and may then resume conduction. The point at which we have most often observed this delay is in the apical region. There are, however, several other regions which are outstandingly prone to develop blocking points. These lie on the ventral surface somewhat laterally, near the base and ventro-laterally, just below the basal angles. Figure 2 shows a striking case of this sort, recorded from a strip which had been severely poisoned with veratrin. Some recovery had taken place

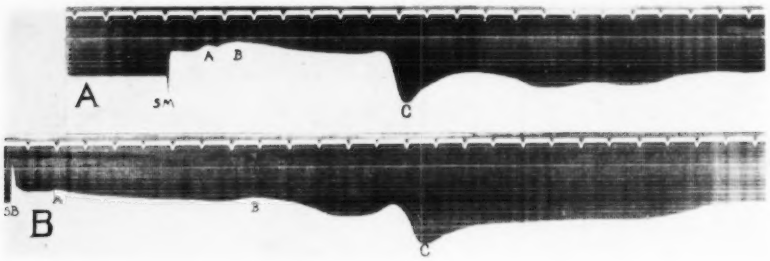

Fig. 2. Capillary electrometer records from strip partly recovered from veratrin poisoning. *SM* and *SB*, record of escape of make and break induction shocks respectively. A and B, peaks of R-wave. C, peak of T-wave.

due to washing with Ringer's solution. Stimulation was made a few millimeters from the proximal lead. A previous record had been taken showing several responses with the same general time relations as those of figure 2B. After a rest of about ten minutes, the records of figure 2 were taken. The response to a make induction shock (fig. 2A) showed quite different time relations from those recorded before, but a closely following break shock gave a response of the form shown as figure 2B, the same as that of the earlier records. Another pair of records was then taken with similar results except that the longer record was in response to the make and the shorter in response to the break shock. A and B indicate the two "major peaks" of the R-wave and C the peak of the T-wave. The times of conduction through the strip are approximately equal to the time intervals between the shocks (SM and SB) and B in the two records shown. The time from B to C is approximately the same in the two cases. The time from A to B, however, changes from about 0.17 second to 1.36 seconds, the delay in conduction being so long that it was clearly visible to the

eye of an observer. The changes in the form of the T-wave may be explained on a basis of the different relative times of beginning recovery in the two electrodes in the two cases (cf. Gilson, 1929, p. 128).

Perfusion preparations under observation also show such blocking effects of veratrin poisoning. The phenomenon is therefore not to be attributed to injury incidental to cutting the strip. The impulses will start, from the point stimulated, then encounter a region of delay or complete block which appears to be the same in successive trials. Obviously the normal path of

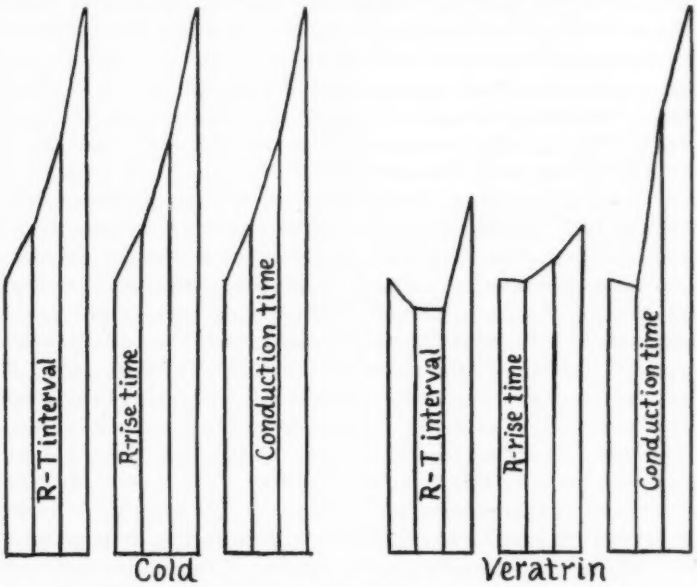

Fig. 3. Diagram of effects of cold and of veratrin upon different aspects of the cardiac muscle reaction. For each unit, increased lowering of temperature or poisoning by veratrin is indicated along the abscissa. Duration of the times plotted from a typical experiment is indicated by the height of the vertical lines.

impulse propagation is upset in such cases and the electrogram becomes highly abnormal. Perfusion with Ringer's fluid will often cause recovery and the operation may be repeated a number of times before block becomes permanent.

Such regions of potential block, so nearly alike in different preparations, lead to the conclusion that at these regions there exist physiologically junction-like points at which it is especially easy to block conduction through the heart. The chief of these lies in the apical region. Indications of the relative discontinuity of the fiber bundles from the right to the

left side of the ventricle in the region of the ventricular apex is indicated in such anatomical works as that of Tandler. It may be that such structural arrangement contributes to the sensitivity of the apical region to block-producing drugs. It is also possible that other anatomical peculiarities of the fiber bundle arrangements may be responsible for the other almost as sensitive points located nearer the basal angles of the ventricle.

The spread of an impulse to all parts of a normal cardiac muscle preparation is good evidence of what is usually referred to as a "physiological syncytium." That blocking, perhaps between the larger fiber bundles, may occur at such specific regions as the sino-auricular and auriculo-ventricular boundaries makes it not improbable that the critical regions occurring within the ventricular mass are of a similar sort. The effects of veratrin in producing sino-auricular block and auriculo-ventricular block are well known and the dissociation of regions of the ventricle has been reported by Kretzer and Seemann (1912).

Discussion. Discussion of the phases of the electrogram should so far as is possible include correlation of the electrical response with the mechanical. It may be well to outline the present status of our knowledge of that relationship in so far as it deals with the completely diphasic electrogram. We have found (Gilson, 1927) that the R-wave appears so soon after stimulation that when a lead is taken from the site of stimulation, the R-wave rises out of the record of the induction shock used. With our present accuracy, then, the latent period of the electrical reaction may be considered as negligible. The onset of the mechanical shortening has been considered by Einthoven (1924–25) to be synchronous with the beginning of the electrical reaction. The time of mechanical latency would by this criterion be placed at zero (or a negligible amount). With a very light isotonic lever and optical registration, we have been unable to achieve this elimination of the latency of the mechanical reaction but a few records show indication of slight movement of the lever which may correspond with the period of increased viscosity described by Fulton (1925) for skeletal muscle. One can readily note beginning of contraction by the time the R-wave has reached its maximum.

The end of the period of contraction at the proximal electrode usually corresponds closely with the *beginning* of the T-wave of a completely diphasic record. Occasional unaccounted for deviations from this relationship occur in our records, though we believe them to be due to the fact that the effective mechanical and electrical records were not obtained from the same point. At this time, or very soon thereafter, there ends the absolute refractory phase. As will be seen by reference, for example to Katz's review (1928) dealing with the T-wave, premature systoles caused by stimuli thrown in during the T-wave are not rare. However, we know of no case in which stimuli applied before the beginning of the T-wave have been

effective in prolonging the systole in progress or in setting up another contraction. The T-wave depends for its form on the time required for conduction through the strip and on the form of the electrical recovery curves of previously activated tissue in the regions from which leads are made. The latter part of the T-wave falls during diastole.

Concerning the general effects of cold and of veratrin, our results are in accord with previous work. The slowing of all phases of the electrogram under the action of cold was to be expected from the records of numerous workers. The veratrin effects are perhaps less clearly indicated by individual papers, but they are in accord with the greater part of the experimental work presented. As most of the published electrograms showing veratrin action have been obtained from frog ventricles, and usually from "monophasic" preparations, the comparison of the phases of the electrogram is not easy. Certain details of the veratrin action, however, meet with general recognition. Among these are the prolonged systole and the interference with conduction from one part of the heart to another. Associated with these is an apparent prolongation of the absolute refractory phase (De Boer, 1914–15). Drury and Love (1926) working with the frog ventricle have shown that under the conditions of their experiments, the *apparent* prolongation of the refractory phase is due to impaired conduction and that the *true* absolute refractory phase is actually shortened by amounts ranging from 0.05 second to 0.2 second. Lewis and Drury (1926) conclude that while the substances used in Drury and Love's experiments (veratrin, quinidine, and strophanthin) do not prolong the actual absolute refractory phase, they do prolong the effective refractory phase and cause the appearance of "condition akin to decrement." We have not attempted measurements of the refractory period with a sufficient degree of accuracy to allow of final conclusions, but we believe that the shortening of the true refractory period found by Drury and Love in the frog corresponds with that period during veratrin poisoning in which we find a marked slowing in conduction but an earlier than normal beginning of the T-wave. With the evidence which is accumulating to indicate a parallelism of durations of the refractory period and the sustained monophasic action potential, it is to be expected that late, severe veratrin poisoning with a delayed start of the T-wave would also cause a prolongation of the true refractory period. If this is true, it seems probable that much of the "condition akin to decrement" may be explained by interferences in conduction perhaps between the fiber bundles. Thus the normal, completely syncytial ventricular mass would be broken up, physiologically, by poisoning the effects of which would not be equal at all points in the ventricle. If the local reaction at the point stimulated recovered more quickly than blocking regions in the vicinity, the apparent decremental effects observed would be found.

We can, by different modes of attack, cause more or less independent

change of several different aspects of the electrogram. These are indicated in a diagram by figure 3. Change in temperature does not (within certain limits) change the height of the R-wave crest but it very greatly changes the time required for the R-wave to attain that crest. The beginning of electrical recovery (which occurs at the time to which we have referred as placed at the end of the monophasic plateau) is delayed by cold, and made earlier by warmth. It is made earlier by slight and later by severe veratrin poisoning. The time required for conduction through the strip may be lengthened at a time when threshold is decreased and duration of the electrical response is shortened, or when both are increased.

The picture of a cardiac response which could be outlined would include the above factors in the chain of events involved in a single response. Stimulation at a given point may result in the breaking down of a polarized resistance with the appearance of a local relative negativity. This depolarization process requires a certain amount of time according to the condition of the tissue. At least by the time this local depolarization is completed, mechanical contraction has commenced. The depolarization persists for a period of time during which application of another stimulus will not cause a second or a prolonged response. It is also (approximately?) during this time that contraction of the cardiac muscle at the point of stimulation is completed. At the end of this period of depolarization, polarization at the point stimulated again begins to develop and continues through the period of electrical recovery. The beginning of this period of electrical recovery comes at (about?) the moment of completion of local contraction. During the period of depolarization at the point of stimulation, the impulse is being propagated at a rate of about 100 mm. per sec. through the strip. This impulse propagation normally occurs *as if* the ventricular tissue were of a completely syncytial nature. A uniform quickening or slowing of conduction may occur. Under certain conditions, however, as under veratrin poisoning, certain regions become especially liable to the development of a high degree of interference with condition and may develop complete blocks before the mass at large shows a greatly depressed conduction rate.

SUMMARY

Lowered temperature causes a decreased excitability and a slowing of all the processes represented in the ventricular strip electrogram. The slowing of these processes may occur in nearly proportionate degrees.

Veratrin poisoning causes at first an increased excitability and a quickening of the processes represented in the electrogram. Further action of veratrin causes a decreased excitability and a slowing of the processes involved. The action on the different processes is not equal, conduction being greatly prolonged at a relatively early stage of poisoning.

The presence of apparently junction-like regions in the ventricular muscle at which blocking of conduction is most likely to occur is described and their significance is discussed.

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PHYSIOLOGY OF THE CORPUS LUTEUM

V. The Preparation and Some Chemical Properties of Progestin, A Hormone of the Corpus Luteum Which Produces Progestational Proliferation

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In previous papers of the present series[1] we have described the preparation and effects of extracts of the corpus luteum. These extracts, when injected into recently spayed female rabbits, regularly bring about a special histological and physiological state of the endometrium, characteristic of early pregnancy, and known by previous experimentation to be due to the corpus luteum. If the animals have been mated the day before castration, the extracts induce normal growth and implantation of the embryos and the animals are maintained in a normal state of pregnancy, even to parturition at full term, in spite of the absence of the ovaries.

We have as yet proposed no name for this hormone of the corpus luteum, referring to it only as a hormone which induces the above-described characteristic effects in the rabbit. In so far as we are acquainted with its physiological behavior, its chief action lies in its ability, by alteration of the endometrium, to aid gestation in the castrated rabbit; and for this reason we wish to propose for it the name *progestin*, i.e., a substance which favors gestation.

The effects in question have been shown not to be due to the substance isolated from follicular fluid by Allen and Doisy (1924), and subsequently purified by them (Thayer, Jordan, and Doisy, 1928; Laqueur, Hart, and de Jongh, 1927, and H. Allan, Dickens et al., 1928). This substance, known as oestrin, folliculin, menformon, amniotin, estrogen, etc., has as its characteristic effects the induction of the oestrous vaginal smear in spayed rats and mice and the causation of growth of the uterus in immature animals. Our papers, in conjunction with the work of Asdell and Marshall

[1] Previous papers of this series are listed in the bibliography as Corner, 1928; Corner and W. Allen, 1929; W. Allen and Corner, 1929; Tatelbaum and Goldstein, 1929. For the extensive literature of ovarian and placental hormones, see E. Allen and Doisy (1927).

(1927), Courrier and Masse (1928), Loeb and Kountz (1928), and Ebhardt (1928), demonstrate that oestrin produces none of the uterine reactions characteristic of early pregnancy, such as progestational proliferation of the rabbit's endometrium or the special sensitization of the guinea-pig's uterus necessary for the production of experimental deciduomata. It seems certain, therefore, that the corpus luteum contains a hormone which is entirely distinct from oestrin in its effects.

In a series of recent notes Hisaw (1929), Hisaw, Meyer and Weichert (1928), and Weichert (1928), have also described extracts of the corpus luteum. These workers used as their chief test the relaxation of the guinea-pig's symphysis pubis, a reaction which normally occurs during pregnancy in this animal. Their extracts when administered to female guinea pigs caused prompt relaxation of the symphysis, and in addition have been found to produce other phenomena characteristic of early pregnancy, such as sensitization of the uterine mucosa, inhibition of ovulation, and alteration of the endometrium (rat).

A third group of workers attempted to obtain potent extracts of the corpus luteum by use of the fact that ovulation does not occur during pregnancy. Extracts of the corpus luteum should be expected, therefore, to produce inhibition of ovulation; and such extracts have been described by Papanicolaou (1926), Parkes and Bellerby (1927), Payne, Van Peenan and Cartland (1928), Brouha and Simonnet (1929), Hisaw, Meyer and Weichert (1928), Macht, Stickels and Seckinger (1929), Gley (1928) and Haterius and Pfiffner (1929). The methods of preparation used by these workers are so divergent in principle, however, that it is not certain, even though the same criterion of activity is used, that they are dealing with the same chemical substance.

The subject is further complicated by the fact that all of the specifically potent ovarian extracts thus far produced have been obtained by similar processes. The earlier workers (Iscovesco, 1912, 1914; Fellner, 1913; Seitz, Wintz and Fingerhut, 1914; Hermann, 1915; and Frank and Rosenbloom, 1915), who used general growth of the immature uterus as their chief criterion of potency, made their extracts by one or another systematic scheme designed first to extract the lipids from the ovarian tissue, corpora lutea or placenta, and thereafter to remove the various known lipids by differential solubilities, fractional distillation, etc.

The procedure of Allen and Doisy for the preparation of oestrin, recently perfected by Thayer, Jordan, and Doisy (1928), is of the same nature. So also is the method which we have described for the preparation of crude extracts of the corpus luteum; and that of Hisaw, as far as made known, is of the same order. We have thus a single method of extraction and purification, yielding at least two distinct substances (oestrin and progestin) according to the original source of raw material. Moreover, in view of the

fact that both these hormones must have been present in the corpus luteum extracts of the earlier workers, before the specific tests were discovered, it is not impossible that still other hormones may be concerned in the different effects described, for example, by Hisaw and ourselves.

For this reason it is important to purify the potent substances as far as possible and to determine their respective chemical characteristics and physiological effects. The present paper describes what has been learned as to the progestin hormone since the appearance of our first paper (Corner and Allen, 1929).

The test (for details, see Corner and W. Allen, 1929) used for every step in the purification and chemical reactions about to be described has been the production of progestational proliferation of the endometrium of the uterus of the spayed adult female rabbit. *It is considered fully positive if, after the daily injection of extract for five consecutive days, the uterus shows on the 6th day a proliferation equal to that of the 8th day of a normal pregnancy or pseudopregnancy.* There is some difficulty in interpreting a complete proliferation according to this standard but in general if the proliferation has been sufficient to cause the survival of some normal embryos it is considered fully positive even though the proliferation may not be quite so complete as that of the 8th day of normal pregnancy. An arbitrary standard of the various degrees of proliferation has been followed, which involves the use of + signs, a + being the smallest amount of typical proliferation which can be recognized and a ++++ being equal to that of an eight-day pregnancy (figs. 1, 2, 3, 4). A +++ or ++++ proliferation is usually sufficient to maintain the embryos when present. *One rabbit unit is the minimum amount necessary to produce a +++ or ++++ proliferation under the specified conditions.* It is not necessary to use mated rabbits since the non-mated, hence non-ovulating, adult female rabbit is equally good for test purposes. In view of the fact, however, that it is not always possible to distinguish the mature from the immature rabbit by size and general appearance, either mated rabbits or ones which were known to be mature by reason of their history have been used.[2]

The accuracy of this test has not been studied quantitatively to determine the degree of variability of the test animals. There is no sharp line of demarcation where it may be said that just below the minimum dose has been given, for as the dose is gradually reduced the degree of proliferation decreases accordingly. Because of this lack of a definite end-point a quantitative study would necessitate the use of a large series of animals. Generally one-half of the minimal dose necessary to produce a ++++ proliferation will give a + or ++ reaction. Therefore, whenever a fraction is tested at a given dosage and found to give no proliferation at all it

[2] For details of the physiology of reproduction in the rabbit and for practical information as to mating, see Hammond and Marshall (1925).

may be assumed with some degree of accuracy that twice that dose is below the minimum necessary to produce +++ or ++++ proliferation. The effect of large doses is also reflected in the degree of proliferation, several times the minimal dose usually producing a proliferation greater than a ++++. Also when the dose is excessive the embryos are occasionally, but not always, abnormally large. In fact embryos $5\frac{3}{4}$ days old have been recovered which were from 2.5 to 3.0 mm. in diameter, twice the normal size for that age.

The protocols of the numerous rabbits used in testing the various fractions need not be given. They have been adult animals castrated about an hour before the first injection was made. A sample segment of the uterus was removed from each at the time of castration to serve as a control. The operative and autopsy specimens of all the uteri have been studied microscopically to determine the exact degree of proliferation. All the animals were autopsied on the day following the last injection, the fifth day after castration. A total of 89 animals has been used. In 41 of these the uterus was fully proliferated; in 12, where the dose was less than minimum, there was partial proliferation; and in 36 where the dose was inadequate or the fraction inactive there was no proliferation. Twenty-six of the 41 which reacted fully were mated animals. In 18 of these there was a total of 70 normal embryos, most of which were obtained from the unoperated horn of the uterus. In this group of 26 there were also 5 with no embryos and 3 with slightly retarded ones. These results add 41 animals with complete proliferation and 18 with normal embryos to the series previously published (Corner and W. Allen, 1929; and W. Allen and Corner, 1929). The remainder of the group of 41 animals which reacted fully was made up of 10 unmated does and 5 mated does which did not ovulate subsequently to mating.

The method of preparation is divided into stages for convenience.

Stage I. *Storage of material.* The corpora lutea of the sow are dissected from the ovary as previously described (Corner and W. Allen, 1929) on the same day that they are received from the abattoir, usually while they are still warm, ground up with an ordinary meat grinder and preserved by adding 2 volumes of 95 per cent alcohol. The material is stored in this condition until ready for use and keeps for at least 10 months with no loss of activity. It is not necessary to use hand-dissected corpora lutea since whole ovaries containing corpora lutea may be used. This latter process requires so much more alcohol for storage and subsequent extraction, however, that it is saving of labor and expense to use hand-dissected tissue. When whole ovaries are used the extract contains more oestrin since ovaries with large corpora lutea also contain many small follicles.[3]

[3] Those who are unfamiliar with the normal appearances and dimensions of the follicles and corpora lutea of the sow are referred to Corner (1921).

Stage II. *Preparation of the crude extract.* The procedure in detail for the preparation of a typical corpus luteum extract is as follows: approximately 1500 grams of tissue, which have been preserved as described, are filtered through a bag or gauze and the residue is divided into two or three equal parts and each part extracted five times with hot alcohol in a Clarke-Bloor extractor (described by Sperry, 1926), each extraction lasting for one hour. About 250 cc. of 95 per cent alcohol are used for each extraction. The mass of tissue in the bags is broken up by kneading between extractions to facilitate the penetration of the alcohol fumes. By this method the lipids are completely removed in a fairly short time and none of the extracted material is heated above the boiling point of alcohol for more than one hour. The extraction alcohol contains a quantity of insoluble debris but this is not filtered off since it is removed by subsequent treatment. The alcohol is distilled off by heat with the aid of the diminished pressure obtained with a good water pump. The extraction and preserving alcohols are distilled in separate flasks since the preserving alcohol is apt to foam by reason of its greater content of water. If a good vacuum is obtained the temperature rarely goes above 60°C. and usually not above 45°C. When the solvent has been almost completely removed, the distillation is stopped to avoid overheating. The residues obtained from the extraction and preserving alcohols are combined and extracted five times with peroxide-free ether (or petroleum ether may be used if desired), 500 cc. being used for the first extraction and 200 cc. for each of the remaining extractions. The ether solutions from the above extractions are combined and the volume reduced to about 100 cc. by vacuum distillation. To this solution 4 volumes of acetone are added together with 10 cc. of a saturated alcoholic solution of magnesium chloride. A heavy flocculent precipitate consisting chiefly of phospholipids is formed and is removed by decanting or centrifuging. The precipitate is redissolved in ether and reprecipitated by acetone five times. After the first or second precipitation the precipitate does not completely dissolve in ether, a fine white precipitate remaining. It is not necessary, however, to remove this precipitate, which consists chiefly of sphingomyelin, since it is eventually removed along with the phospholipids by the discarding of the precipitate obtained by the addition of acetone.[4] The acetone-ether solutions are combined and the solvents removed by vacuum distillation on the steam bath. A heavy oil is obtained which is removed from the distilling flask to a centrifuge tube with the aid of about 50 cc. of ether. The solution is centrifuged, thus removing most of the water which is carried through the above solvents together with any

[4] The phospholipid precipitate may be transferred to a Bloor extractor and extracted three or four times with acetone, each extraction lasting for about 1 hour. If this is done however it is necessary to add a small amount of $MgCl_2$ solution and cool to remove the phospholipids dissolved in the hot acetone.

ether-insoluble material. The ether layer is poured or pipetted off and the ether is then removed by a current of warm air. The resulting oily mixture is semi-fluid and may be administered without the addition of a vehicle, providing it is made fluid by immersing the container in warm water for a short time. The yield is about 30 grams, representing 20 grams per kilogram of fresh tissue.

The yields of active substance from extracts prepared in this way have been quite constant, averaging about 50 rabbit units per kilogram of fresh raw material (table 1). In most cases about 500 milligrams are ample for one rabbit unit (100 mgm. per day for five days).

The stability of this crude preparation is limited, however, since several preparations have shown a loss of most of their activity in a month's time. For example, when one extract was new 500 mgm. were sufficient for 1 rabbit unit but after standing 10 days' time, twice that dose was barely

TABLE 1

Amount of hormone from dissected corpus luteum

PREPARATION NUMBER	TISSUE EXTRACTED	YIELD PER KILOGRAM OF FRESH TISSUE	MINIMAL DOSE	RABBIT UNITS PER KILOGRAM
	grams	*grams*	*grams*	
43	1,512	20.1	0.56	>36 and <72
46	1,506	21.2	0.56	>38 and <76
48	1,483	20.5	0.45	45
52	2,004	25.4	0.58	>44 and <56
54	1,991	18.6	0.36	52
56	1,443	22.1		
57	1,857	23.0	0.45	52
60	1,500	24.3	0.45	54
63	897	18.2	0.45	40

sufficient. Another preparation was totally inactive after standing 1 month.

Since this crude oil seemed so easy to use for physiological purposes its stability in various solvents was tested. Alcohol and acetone in equal parts was first tried since this mixture dissolved the crude oil quite readily. Surprisingly enough, however, the one preparation tried lost most of its potency in a short time. The minimal dose before storing in this solution was 500 mgm. but after standing for 15 days 1 gram gave no reaction whatsoever. Alcohol (95 per cent) was then tried even though it was a relatively poor solvent. About 550 cc. alcohol were added to 20 grams of the crude extract, the solution was warmed until the fat all dissolved, and then cooled. Much of the fat and cholesterol separated out but this did not seem to impair the keeping qualities. Several preparations have been stored in alcohol in this manner and none have shown any appreciable

loss even after a month's standing. As a result of this finding the procedure now used is to store all preparations in alcohol when it is necessary to keep them for any length of time. When a large amount of extract is desired a good procedure is to carry the purification through stage II and re-dissolve the extract in alcohol; then, when the material is needed, to distill the alcohol from enough extract to last from 5 days to one week, keeping the stock extract in solution. The absolute stability in ethyl ether and petroleum ether has not been investigated but there is no apparent loss after standing two or three days in these solvents.

Stage III. *Removal of the neutral fats.* The next step in the purification consists in the removal of a large amount of the neutral fat and cholesterol by freezing from methyl alcohol. In a typical preparation the procedure is as follows: 30 grams of the crude material are shaken up with 200 or more cubic centimeters of boiling methyl alcohol. The larger part does not dissolve, but by shaking and stirring it can be broken up sufficiently to allow good penetration of the solvent. After thorough agitation the mixture is transferred to 75 cc. centrifuge tubes and is kept at −5°C. for 12 hours or more. The insoluble material is then driven to the bottom of the tubes by centrifugation, the supernatant liquid decanted and replaced in the ice box, and the insoluble material again shaken up with 200 cc. of boiling methyl alcohol. This is then kept at −5°C. as in the first freezing and the process repeated until the methyl alcohol after being cooled extracts no more coloring matter. When this freezing has been repeated about 5 times, the fat at the bottom of the tubes is lemon yellow and the cholesterol crystals are perfectly white. If any precipitate forms on cooling in the alcohol decanted from the various extractions, it is removed by filtering through cotton, the alcohol from the first extraction being filtered first, that from the second next, etc. By filtering in this order the precipitate is washed last by cold alcohol which contains practically none of the active substance. The methyl alcohol may be concentrated to about 100 cc. and the freezing process repeated if it is desired to remove as much of the cholesterol as possible from this solvent. It can not be removed completely by this additional freezing, hence it seems inadvisable to reduce the volume too much, a small volume being so much more difficult to handle. The insoluble material, amounting to about 15 grams in this case, is discarded since it is inactive. The methyl alcohol is then distilled off in vacuo to dryness, the residue taken up in petroleum ether (purified over concentrated sulphuric acid and redistilled) and washed with 0.01 N sodium hydroxide solution until the washings remain alkaline to litmus, thus removing as soaps the fatty acids present. The ether solution is then washed a few times with 0.01 N hydrochloric acid solution and then with distilled water. The petroleum ether is distilled off and the residue taken up in ethyl ether. This is then washed several times with distilled water.

It is advisable to wash the residue in both ethyl ether and petroleum ether since there is some inactive material soluble in petroleum ether which is insoluble in ethyl ether and vice versa. The ether is distilled off and the residue thoroughly dehydrated by adding about 50 cc. of absolute alcohol and then distilling it off in vacuo. By this process the water present, which is inevitably carried along in the ether, is removed, a necessary step to assure complete solution of the residue in Mazola (commercial maize oil). The residue after removing the alcohol amounts to 10 grams, representing 6.6 grams per kilo.

When this step in the purification is carried out as described there is very little, if any, loss in the active substance and the minimal dose is reduced from 500 mgm. to about 130 mgm. By carrying the freezing process farther and by more careful and prolonged washing of the ethereal solution it may be reduced to 60 or 70 mgm. It is a brown viscous oil containing much cholesterol. Because of the small amount necessary for a rabbit unit this preparation before injection has always been dissolved in a convenient amount of Mazola, solution being complete if the oil is thoroughly dehydrated as described above.

The stability of this preparation is apparently complete in ethyl alcohol for at least a month. It is not stable, however, when dissolved in Mazola, one preparation having lost its entire potency after 24 days in this medium. Because of this deterioration in Mazola it is always necessary, when standardizing fractions dissolved in Mazola, to give the minimal dose when the preparation is first made. To do this the general practice has been to give the theoretical minimal dose to one rabbit and twice that amount to another rabbit at the same time. It would seem wiser perhaps to give twice the minimal dose to a rabbit and if this proved active to reduce the dose on the next rabbit. This is not trustworthy, however, because of the deterioration in Mazola. The same method of standardization is also necessary for the crude extract obtained from the end of stage II, since that preparation also shows deterioration with standing.

Stage IV. *Separation from cholesterol.* The effective separation of the cholesterol from the active substance has not met with a great deal of success. A similar method to the one used by Thayer, Jordan and Doisy (1928) for the separation of cholesterol from oestrin, has been tried but without as good results as they found. If a 70 per cent ethyl alcohol solution containing the hormone and cholesterol is extracted with petroleum ether there is considerable loss of potency to the petroleum ether, as high as 40 per cent in some cases, if a sufficient number of extractions is made to remove completely the cholesterol from the 70 per cent alcohol. The minimal dose of the active substance remaining in the alcohol is reduced to about 30 mgm., however, and contains so little cholesterol that no color reactions for it can be obtained. In other cases there has been consider-

able loss of the active substance in this process, only about 50 per cent of the hormone being recovered from both the petroleum ether and alcohol fractions. The only result gained from this removal of the cholesterol is that the hormone may be obtained in an aqueous medium which seems to be a fairly stable colloidal suspension, by merely distilling off the alcohol from the 70 per cent solution. An insufficient number of aqueous preparations of the hormone have been made to warrant any statement whatsoever regarding its stability in this condition. The pH of the few extracts made by this method has been about 4.8.

The detailed procedure is as follows for a given experiment: 10 grams of the oily residue from the preceding stage, containing about 75 rabbit units, are dissolved in 225 cc. of 70 per cent ethyl alcohol. Seventy-five cubic centimeters of petroleum ether (purified over concentrated sulphuric acid and redistilled) are added, the mixture shaken thoroughly, and the ether layer removed. The alcohol is extracted 5 times or more in this manner, 2 cc. being removed after the third extraction from each of the petroleum ether layers to be tested for cholesterol by the Libermann-Burchard color reaction. Usually after the fifth extraction 2 cc. of the petroleum ether will give no color test. The petroleum ether is removed by distilling in vacuo and the residue transferred to a weighed vial with the help of a little ethyl ether. The ether is then removed by warming in vacuo. The yield is 9.2 grams and contains about 18 rabbit units. The 70 per cent alcohol fraction is distilled to dryness in vacuo. Fifty cubic centimeters of absolute alcohol are then added, and this removed in vacuo. The yield is about 2 grams and is active in 40 mgm. doses, giving a potency of 50 rabbit units. If it is desired to have an aqueous solution from the alcohol fraction, the distillation of the 70 per cent fraction is stoppped when the alcohol is all removed and the aqueous remainder is made up to any standard volume convenient for injection.

CHEMICAL REACTIONS OF THE HORMONE. *Saponification. a.* Ten grams of the crude product obtained from stage II, containing 20 rabbit units of the hormone, were dissolved in 200 cc. of normal sodium ethylate and heated on the steam bath until the alcohol had all evaporated (6 hours). The non-saponifiable matter was extracted with ethyl ether, the ether layer washed several times with distilled water and the ether then distilled off in vacuo. The yield obtained was 2.84 grams. One and forty-two hundredths grams were injected and were totally inactive, hence none of the 20 rabbit units were recovered.

b. Fifty-two hundreths gram of the product, obtained from stage III, containing from 3.3 to 4 rabbit units, was dissolved in 15 cc. of 2 per cent alcoholic potassium hydroxide and heated for 2 hours on the steam bath, alcohol being added to replace that which was lost by evaporation. At the end of 2 hours the alcohol was distilled off and the residue extracted

with ethyl ether. This was next washed several times with distilled water. The ether was then distilled off. The yield obtained was 0.21 gram. Eighty-four milligrams, 1.3 times the theoretical minimum dose, were injected and found to be entirely inactive.

c. Fifty-two hundredths gram from the same extract as the above, containing from 3.3 to 4 rabbit units, was dissolved in 10 cc. of 95 per cent alcohol. Enough alcoholic potassium hydroxide was added to make the solution faintly alkaline to phenolphthalein and then allowed to stand for 5 days at room temperature. The pH at the end of the second day was 7.8 (beyond pH 10 at the beginning of the process) and remained at that figure. After five days the alcohol was distilled off and the residue extracted thoroughly with ethyl ether. The ether was distilled off, leaving a residue of 0.328 gram. This was active when given in a 164 milligram dose, hence 2 rabbit units were recovered and the minimum dose was not obtained. The ether-insoluble material in this case was tested and found to be inactive.

d. Two and nine-tenths grams of the crude preparation obtained from stage II, containing 6.4 rabbit units, were dissolved in 200 cc. of 2 per cent alcoholic potassium hydroxide (95 per cent) and heated on the steam bath until the solvent was all evaporated. The residue was extracted with ethyl ether and the ether was washed several times with water. The soaps and washings were injected and found to be inactive. The ether was distilled to dryness, leaving a residue of 1.3 grams. This was injected into one rabbit but was totally inactive. Hence none of the 6.4 rabbit units were recovered in either fraction.

e. Two and four-tenths grams of crude product (stage II) containing 5.3 rabbit units were dissolved in cold 2 per cent alcoholic potassium hydroxide. This was immediately distilled off in vacuo (temperature below 30°C. throughout) and the residue extracted with ether. This was washed several times with water. The ether was then distilled off leaving a residue of 1.9 grams. Five-tenths gram, 1.3 times the theoretical minimal dose assuming no loss, was given and found to be entirely inactive.

Stability in acid solutions. *a.* Two and seven-tenths grams of the crude preparation (stage II) containing 6 rabbit units were dissolved in 100 cc. of glacial acetic acid and heated on steam bath (90°C.) for two hours. This was cooled and neutralized with concentrated sodium hydroxide to a faint blue with litmus. This was filtered and the filter paper extracted with alcohol and ether. These solvents were distilled off in vacuo leaving a residue of 2.2 grams. Forty-four hundredths gram gave a complete proliferation. Hence, at least 5 rabbit units were recovered, indicating little or no loss.

b. Two and six-tenths grams of the crude preparation (stage II) 5.8 rabbit units, were dissolved in acid alcohol (2 per cent HCl) and boiled

for 2 hours. The alcohol was then distilled off and the residue extracted with ether. The ether solution was washed until free of hydrochloric acid. Upon distilling off the ether a yield of 2.6 grams was obtained. Forty-five hundredths gram gave a complete proliferation, hence 5.8 rabbit units were recovered, indicating no loss of activity.

c. Two and five-tenths grams of crude preparation (stage II) containing 5.5 rabbit units were heated for 2 hours in 0.5 N hydrochloric acid (aqueous) at 90°C. The aqueous solution was then extracted with ether and the ether solution washed with water. The solution was distilled to dryness, leaving 2.3 grams. One gram gave a complete proliferation. Hence 2.3 rabbit units were recovered and the minimum dose was not ascertained.

Oxidation by hydrogen peroxide. a. Two and five-tenths grams of crude preparation (stage II) containing 5.5 rabbit units were dissolved in 100 cc. of alcohol and 10 cc. of hydrogen peroxide (commercial grade) added. This was allowed to stand at room temperature for 4 days. On the 4th day 2 cc. liberated iodine immediately from an acid potassium iodide solution. The alcohol was distilled off in vacuo. Yield 3.2 grams. This preparation was very hard to liquefy by immersing the container in warm water. One gram was active and 0.5 gram was inactive. More than 3.2 and less than 6.4 rabbit units were recovered, indicating little if any loss.

Stability toward heating. a. Five hundred and fifty-eight thousandths gram of the oily material from stage III, containing from 3.3 to 4 rabbit units, was heated in vacuo at 30 mm. at 100°C. for 2 hours. After this treatment a dose of 280 milligrams gave a complete proliferation.

b. Five hundred and nineteen thousandths gram of the same extract was heated in an atmosphere of nitrogen at 100°C. for 2 hours; 260 milligrams gave a complete proliferation.

c. Four hundred and thirty-nine thousandths gram of the same extract was heated in an atmosphere of air for 2 hours at 100°C. Two hundred and seventy miligrams gave a complete proliferation and 135 milligrams a partial proliferation.

The active substance, therefore, is not rapidly destroyed by heating at 100°C.

From these reactions it is obvious that the active substance is destroyed by saponification with sodium ethylate and alcoholic potassium hydroxide but is not destroyed by standing in alcohol at a pH of 10 for several days. It is not destroyed, however, by heating in glacial acetic acid for 2 hours at 90°C., nor by heating in 0.5 N hydrochloric acid solution for 2 hours at 90°C., nor by boiling in acid alcohol (2 per cent HCl) for 2 hours. It is also relatively thermostable. Oxidation by hydrogen peroxide in alcoholic solution for several days causes little or no loss in activity.

The lack of stability of this hormone (progestin) to alkaline hydrolysis is in direct contrast to oestrin. Doisy (Ralls, Jordan and Doisy, 1926) states that oestrin may be saponified with mild alkali and Laqueur (1926) with 26 per cent potassium hydroxide, and Allan, Dickens et al. (1928) with 20 per cent potassium hydroxide, without loss. To verify these statements oestrin was prepared from follicular fluid by the method of Thayer (1928). The minimal dose for a rat unit was 0.048 mgm. Eight milligrams, containing 166.6 rat units of oestrin, of this product were dissolved in 200 cc. of 2 per cent alcoholic potassium hydroxide and heated on steam bath for 2 hours, sufficient time to evaporate all of the alcohol. The residue was extracted with ether, the ether washed with water and finally distilled to dryness. The residue was taken up in 70 per cent alcohol and the alcohol distilled off, leaving an aqueous solution. This solution contained 166.6 rat units, the same amount as before treatment with 2 per cent potassium hydroxide.[5]

This result, together with the statements of Doisy, Dodds and Laqueur, seems sufficient to warrant the statement that oestrin is stable to saponification.

This difference in reaction to alkalis between oestrin and progestin seems to be definite; a reaction which apparently points to a chemical difference between the two hormones. However, this is the only clean-cut difference thus far encountered.

Discussion. The method of preparation is very similar to that used originally by Herrmann (1915). He is practically the only investigator who shows illustrations which indicate that an extract produced progestational proliferation. With some of his preparations he obtained proliferation but it seems more than likely that he did not realize the significance of this result, for he says that in the majority of cases he was guided by the general growth effect and the engorgement of the external genitalia, both responses which are now known to be produced readily by oestrin. These statements together with the fact that he considered the human placenta as rich in the hormone as the corpus luteum make it practically certain that he did not differentiate between the effects of oestrin and the proliferation due to progestin. Because of this unreliability of his test it is not possible to compare critically his method with the one described. He extracted the minced dried tissue with ether, removed the phospholipids by acetone, the fats by freezing from methyl alcohol, and the cholesterol by freezing from ethyl alcohol. The final product was then distilled in high vacuum (0.06 mm). This method of preparation certainly will give

[5] Only 4 rats were used in assaying the strength of these oestrin preparations, but the minimal dose was considered the smallest dose which produced a typical oestrus smear in all four of the rats. A dose which produced oestrus in 3 of the 4 was considered subminimal.

a product containing much oestrin when prepared from the placenta and will also give a preparation containing progestin when prepared from corpus luteum tissue. (High vacuum distillation has not been tried here, hence progestin may or may not be destroyed by that process.)

Fellner (1913) seems also to have elicited proliferation in some of his immature rabbits with extracts of placenta (his fig. 2). He recognized that this was a similar effect to that demonstrated by Bouin and Ancel (1910) to be due to the corpus luteum. The method used by him involved

Fig. 1 Fig. 2 Fig. 3 Fig. 4

Fig. 1. Control section of uterus of rabbit ×49, 18 hours after mating. ×7. No proliferation. Compare with figure 4.

Fig. 2. Section of uterus of ×167, showing ++ proliferation as a result of 5 days' treatment with one-half the effective minimal dose of corpus luteum extract. ×7.

Fig. 3. Section of uterus of ×185, showing +++ proliferation obtained from 5 days' treatment with a minimal dose of corpus luteum extract. ×7.

Fig. 4. Section of uterus of ×148, showing ++++ proliferation obtained from 5 days' treatment with corpus luteum extract. ×7. Compare with figure 1.

only the extraction of the tissue with alcohol, the evaporation of the alcohol, and the suspension of the remaining material in saline for injection. This work likewise need only be commented on since it has been shown (Corner and Allen, 1929) that the immature rabbit is an unreliable test for the corpus luteum hormone. One of the photographs shown by Frank and Rosenbloom (1915, fig. 2, section 4) likewise hints at a slight degree of proliferation. The placenta therefore may contain some of the hormone under certain conditions but in no case in which the adult animal was used as a test have we obtained proliferation with placental extracts.

Hisaw in a series of preliminary notes has shown that extracts of corpus luteum may be prepared which relax the symphysis pubis of the female guinea pig. This relaxation is a normal concomitant of pregnancy in this animal and he has shown that it can not be produced by oestrin. The detailed method of preparation is as yet unpublished but he states (1929) that it is extractable with acid alcohol but not with ether, acetone, petroleum ether or chloroform, and that it is destroyed by strong bases. The lack of stability with alkalis and the stability in acid alcohol may indicate similarity with progestin. The progestin is soluble in ether, acetone petroleum ether and chloroform but it may not be extractable by these solvents since they all are notoriously poor solvents for the lipid extraction of fresh tissue. Hisaw also states that the potency of his extracts is readily destroyed by heat, a fact which indicates a dissimilarity since progestin is not destroyed by a temperature of 100°C. Until a complete procedure for preparation is published by him it must be admitted however that at present there is no striking chemical difference between the two substances, even though his chief test is an entirely different one.

The relationship between progestin and those extracts which inhibit oestrus is not apparent as yet. The method used by Parkes and Bellerby (1927) and Haterius and Pfiffner (1929) for the preparation of a substance inhibiting oestrus is quite similar to that described for progestin. Payne, Van Peenan and Cartland (1928) (method of preparation given by G. J. Cartland, and M. C. Hart, 1925) however claim to have inhibited oestrus with the non-saponifiable fraction from corpus luteum. This method certainly destroys progestin. Gley (1928) and Macht (1929) have used very divergent methods, hence it can only be conjectured that oestrus may be inhibited by non-specific substances.

SUMMARY

An improved procedure for the preparation of a hormone of the corpus luteum which produces progestational proliferation is described, and evidence is presented to show that it is destroyed by alkalis, thus differentiating it chemically from oestrin. The purified product is not destroyed by mild acid hydrolysis nor is it associated with cholesterol color-reactions. It is relatively stable to heat and mild oxidation. Data are given regarding the yields of active substance. The oily preparations deteriorate rapidly but they may be kept in ethyl alcohol for long periods of time without loss. The name "progestin" is proposed for this specific hormone of the corpus luteum.

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THE BASAL METABOLISM AND VITAL CAPACITY OF SYRIAN WOMEN

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Interesting and significant results have been obtained during the past few years on racial variations in basal metabolism. Most of the comparative studies have been made in oriental, tropical and subtropical regions and the majority of the findings indicate a lowered metabolic rate in individuals living in these places. Both DuBois (1927) and Benedict (1928) have stated that the normal prediction standards are somewhat too high even for American men and women.

de Almeida (1924), working in Brazil, obtained results showing that the basal metabolism of the Brazilian whites was −24 per cent below the Harris and Benedict prediction standards. This work has been questioned by different investigators from time to time but, to the author's knowledge, de Almeida's results have not been proven erroneous. Earle (1922) stated that the metabolic rate of the Chinese was set at a lower level than that of western races and Yano (1920) and Takahira (1924) both found the metabolism of Japanese to be lower than the standards. MacLeod, Crofts and Benedict (1925a, b) working on Chinese and Japanese women students attending an American college, found the average basal metabolism of their nine subjects to be −10.4 per cent below the Harris and Benedict standards. On the other hand Okada et al. (1922–1926) found the level of Japanese metabolism to be practically the same as that of western races and they concluded that the standards were utilizable for Japanese subjects.

Turner (1926) working on a mixed group of Near Eastern subjects found that Armenian men and women gave average metabolism values very close to the Aub and DuBois standards. Other Near Eastern races gave lower values. Outside of a group of nearly forty Armenian women most of the other subjects in this study were males from various Near Eastern countries. A small group of ten women consisting of two Jewesses, two Palestinian arabs, two Egyptians and four Syrians were studied. This small mixed group of women gave an average basal metabolism value about 6.0 per cent below the Aub and duBois standards. Control studies were made on young American and English men residents of the Near East. Heinbecker (1928) found the metabolism of Eskimos in the Baffin Bay district

to average plus 33.0 per cent above the normal standards. Wardlaw and Horsley (1928) studied the metabolism of a group of Australian Aborigines and found them to give low average values.

Steggerda and Benedict (1928) using the portable field apparatus developed at the Carnegie Nutrition Laboratory found the metabolism of Jamaica browns to be practically normal, averaging −3.4 per cent, while the Jamaica blacks were slightly lower, averaging −5.4 per cent. It is of interest to note that these subjects were all hard working men carrying heavy loads as their routine labor. Williams and Benedict (1928) using the same type of apparatus studied the metabolism of the Mayas in Yucatan and controlled their observations by studies on whites living in the same district. They found the male white controls to have an average basal metabolism of −3.4 per cent, the women −2.4 per cent while thirty male Mayas gave an average value of +5.2 per cent. They also made observations on the basal metabolism of the investigators before, during and after the sojourn in Yucatan.

When the senior author (Turner) made the original investigations on basal metabolism in the Near East it was a very difficult problem to obtain normal Syrian women as subjects. A few Christian Syrian women were available but there were no means of getting into contact with normal Moslem women. It would have been easy enough to gather data on any number of pathological Moslem women subjects attending the American University Clinics and Dispensary, but the performance of such tests upon perfectly normal young women was looked upon with considerable skepticism by prospective subjects. The junior author, a young woman medical student in the American University, became interested in the problem and was able to furnish the necessary subjects. Owing to the orthodox views regarding the exposure of a woman's face, held by some of the Moslem subjects, it was necessary that Miss Aboushadid perform the tests alone in several instances.

Twenty-eight young Syrian women between the ages of 16 and 33 were studied in this series. The average age was 22. A group of control studies was made on seven American, English and German women living in Beirut. All of the controls had lived in the Near East for at least two years, two of them having resided there over ten years and two others over five years. All but four of the Syrian women were typical subjects having dark hair and eyes with a tendency toward olive skins. They live a relatively quiet life doing little in the way of manual labor.

All of the tests were performed with a Krogh metabolimeter which was carefully checked from time to time to insure against leaks. The Aub and DuBois standards were used. At least two tests were performed on each subject. Two complete records were taken with each test. All tests were made between seven and ten in the morning. No breakfasts were taken

by the subjects on the mornings of their tests. A preliminary rest period of from twenty to thirty minutes was allowed before the tests were started. Each individual was shown the apparatus and given some information as to

TABLE 1

Vital capacities of Syrian women

SUBJECT	SURFACE AREA	CALCULATED NORMAL VITAL CAPACITY	ACTUAL VITAL CAPACITY	VARIATION FROM NORMAL	
	sq. m.			*cc.*	*per cent*
M. Ka	1.45	3,001	2,950	−51	−1.7
A. T.	1.50	3,105	2,600	−505	−16.2
N. K.	1.42	2,939	2,600	−339	−11.6
J. S.	1.55	3,208	2,500	−708	−22.1
A. S.	1.58	3,270			
M. Kh.	1.55	3,208	2,450	−758	−23.7
S. K.	1.57	3,249	2,750	−499	−15.4
Su. K.	1.45	3,001	2,550	−451	−15.0
W. K.	1.48	3,063	2,500	−563	−18.4
M. A.	1.66	3,436	3,100	−336	−9.8
S. Khu.	1.57	3,249			
Al. A.	1.50	3,105	3,000	−105	−3.4
B. K.	1.59	3,291	3,000	−291	−9.0
S. Mi.	1.43	2,946	2,600	−346	−11.7
Ed. A.	1.49	3,084	3,000	−84	−2.8
Eu. A.	1.56	3,229	3,100	−129	−4.0
M. H.	1.85	3,829	2,800	−1029	−26.9
K. A.	1.49	3,084	3,000	−84	−2.8
Z. T.	1.48	3,063	3,100	+37	+1.2
R. A.	1.39	2,877	2,500	−377	−13.1
N. H.	1.74	3,601	3,400	−201	−5.5
A. Kh.	1.61	3,332	2,900	−432	−12.9
R. Ka.	1.49	3,084	3,700	+616	+20.5
W. Ka.	1.60	3,312	3,500	+188	+5.7
A. Ka.	1.50	3,105	3,250	+145	+4.6
W. M.	1.69	3,498	2,900	−598	−17.4

Average deviation from normal calculated vital capacity for the entire group ..... −11.5 per cent

Leaving out subjects Z. T, R. Ka., W. K. and A.Ka. as not typical Syrians, the average deviation from the normal calculated vital capacity for the remainder of the group ..... −12.1 per cent

the principle underlying the test. Whenever possible we allowed a subject to observe a test being performed on someone else. This little preliminary was quite valuable as it quieted any fears or suspicions before hand. All of the subjects coöperated in a very satisfactory manner. Vital capacity and blood pressure readings were recorded for each subject. It is desirable

to point out that the studies were made in the summer with the temperature hovering around 27°C., the barometer around 758 mm. Hg and the humidity around 70 per cent as the result of the proximity to the Mediterranean Sea.

The vital capacity of the Syrian subjects averaged lower than that of the control group, both groups averaging below the normal values. Normal values were predicted according to the formula 2.07 × sq. m. surface area (MacLeod, 1927). The vital capacity measurements are shown in tables 1 and 2. The average vital capacity for the entire Syrian group was −11.5 per cent. When subjects Z. T., R. Ka., W. Ka. and A. Ka. are eliminated as not typical Syrian subjects this value falls to −12.1 per cent. The average vital capacity of the control group was −7.1 per cent. One

TABLE 2

Vital capacities of control subjects

SUBJECT	SURFACE AREA	CALCULATED NORMAL VITAL CAPACITY	ACTUAL VITAL CAPACITY	VARIATION FROM NORMAL	
	sq. m.			*cc.*	*per cent*
J. S.	1.64	3,394	3,100	−294	−8.6
H. P.	1.45	3,001	3,300	+299	+10.0
M. D.	1.61	3,332	3,100	−232	−6.9
E. S.	1.60	3,312	3,300	−12	−0.3
B. D.	1.62	3,353	3,150	−203	−6.0
E. R.	1.62	3,353	2,700	−653	−19.5
A. D.	1.76	3,643	2,950	−693	−19.0

Average deviation from normal calculated value..... −7.1 per cent

Subjects A. D. and E. S. had lived in Near East over 10 years.
Subjects E. R. and B. D. had lived in Near East over 5 years.
Subjects J. S., H. P. and M.D. had lived in Near East 2 years.

fact came out of this vital capacity and basal metabolism study that is worth noting as being of some significance in helping to explain some of these racial variations. In table 1 subject Z. T. was the daughter of a Syrian father and an English mother. She was a typical blond with blue eyes, fair skin, light hair and could easily have passed as an Anglo-Saxon. Her vital capacity was +1.0 per cent over the calculated value. Subjects R. Ka., W. Ka., and A. Ka. were sisters. They were daughters of Syrian parents. All three of them were light haired and had blue eyes. They certainly were not pure Syrian Arabs. They all gave a high vital capacity and a relatively high basal metabolism. Since there is a mixture of races as a results of Crusader times in the Near East it is possible that these three women have a mixed hereditary background. These fours subjects

were left in the series even though they do not represent typical Syrians, because they throw an interesting sidelight as to one of the reasons for

TABLE 3

Showing the basal metabolism of Syrian women

SUBJECT	AGE	HEIGHT	WEIGHT	TEMPERATURE OF APPARATUS	BAROMETER READINGS	PULSE	BLOOD PRESSURE	BASAL METABOLISM COMPARED TO AUB AND DUBOIS STANDARDS	DATE
		cm.	*kgm.*					*per cent*	
M. Ka.	25	159.0	46.8	25.0	760.8	62	95/70	−13	6/21/29
A. T.	20	156.0	52.5	25.0	760.9	68	110/75	−15	6/17/29
N. K.	18	152.0	48.0	25.8	759.0	64	100/70	−9	6/25/29
J. S.	25	154.2	57.5	25.9	757.8	64	110/70	−15	6/26/29
A. S.	33	158.2	57.5	25.8	758.6			−11	6/27/29
M. Kh.	27	151.0	58.5	26.8	757.0	72	98/62	−15	7/ 5/29
S. K.	19	153.5	59.0	27.0	755.8	78	110/85	−14	7/ 8/29
Su. K.	18	151.5	50.0	27.0	755.4	66	100/75	−7	7/ 8/29
W. K.	20	154.0	52.7	27.2	758.0	60	95/65	−19	7/10/29
M. A.	17	167.0	58.7	26.7	759.0	68	110/68	−18	7/17/29
S. Khu.	21	153.5	59.3	26.2	759.0	76		−10	7/18/29
Al. A.	24	163.0	47.0	26.5	758.6	66	100/60	−11	7/24/29
B. K.	25	155.5	60.7	27.0	757.4	68	100/75	−17	7/24/29
S. Mi.	25	152.0	47.3	26.5	758.6	74	100/60	−11	7/24/29
Ed. A.	22	161.6	47.8	27.0	759.5	72	110/78	−19	7/25/29
Eu. A.	20	156.0	57.2	27.0	759.5	64	105/75	−19	7/25/29
M. H.	30	168.0	75.0	27.0	759.2	68	115/80	−14	7/27/29
K. A.	25	150.0	55.5	27.3	757.7	64	100/75	−15	7/30/29
Z. T.	19	161.5	47.3	27.3	757.7	74	101/70	−8	7/30/29
R. A.	28	153.0	45.5	27.3	757.7	62	98/60	−5	7/30/29
N. H.	20	158.0	73.6	28.0	758.4	58		−15	8/ 5/29
A. Kh.	21	157.0	62.0	27.3	757.9	76	102/70	−13	8/10/29
R. Ka.	16	157.0	52.0	27.9	759.4	74	95/60	−9	8/12/29
W. Ka.	18	156.5	61.0	28.0	755.8	64	98/60	−3	8/14/29
A. Ka.	20	154.0	52.7	28.0	755.8		95/60	−2	8/14/29
W. M.	18	161.0	64.7	27.9	759.4	76	105/72	−8	8/16/29

Average deviation from Aub and DuBois Standards for entire Syrian group..... −12.1 per cent

Leaving out atypical subjects Z. T., R. Ka., W. Ka., A. Ka., the average deviation from the standards becomes..... −13.3 per cent

racial variations. Thus there is the possibility of an inherited metabolic level that may be independent, to a certain extent, of environmental

conditions. We are certainly still in ignorance as to the cause of the racial differences in metabolism and vital capacity though it would seem that climate, diet, general activity and other factors must play some rôle.

Blood pressure records were all taken with the subject in the sitting posture a short time after completing the metabolism test. The highest blood pressure reading was 115/80 and the lowest 95/60 in the Syrian group. The average systolic reading for the Syrian women was 102.6 and the average diastolic reading 69. The average systolic reading in the control group was 104 and the diastolic reading 68 showing no appreciable difference between the two groups. (See tables 3 and 4.)

TABLE 4

Showing basal metabolism of control subjects

SUBJECT	AGE	HEIGHT	WEIGHT	TEMPERATURE OF APPARATUS	BAROMETER READINGS	PULSE	BLOOD PRESSURE	BASAL METABOLISM COMPARED TO AUB AND DUBOIS STANDARDS	DATE
		cm.	*kgm.*					*per cent*	
J. S.	30	160.7	61.2	26.8	756.8	75	108/70	+3.0	8/6/29
H. P.	22	151.6	50.3	27.0	756.7	60	104/74	−1.0	8/6/29
M. D.	40	159.0	58.2	27.8	755.8	66	100/65	−15.0	8/7/29
E. S.	38	166.7	55.0	27.8	755.8	56	98/60	−4.0	8/7/29
B. D.	24	168.0	55.0	27.0	759.4	60	103/68	−12.0	9/3/29
E. R.	41	167.6	56.0	27.5	758.0	72	104/66	−7.0	9/3/29
A. D.	49	160.0	72.6	27.2	756.0	68	111/70	−6.0	9/5/29

Average deviation from Aub and DuBois standards..... −6.0 per cent

Pulse rates (tables 3 and 4) in the Syrian subjects gave values so closely approximating those obtained in the control group that they may be considered as normal. The lowest pulse rate in the Syrian group was 58 and in the control group 56. The highest pulse rate in the Syrian group was 76 and in the control group 75. The average pulse rate for the Syrian group was 68 and for the control group the value was 65.

Basal metabolism values were definitely lower in the Syrian subjects (table 3) than in the control group (table 4). The highest value obtained for the Syrians was −2.0 per cent and the lowest value was −19.0 per cent, the average value being −12.1 per cent. All metabolism readings were made to the nearest unit, fractions being eliminated. When the four subjects Z. T., W. Ka, R. Ka and A. Ka. are eliminated the value for the Syrian group falls to −13.3 per cent. It should be noted that these four subjects give metabolism values higher than the average of the

Syrian group, the average of the four being −5.5 per cent or practically that of the control group. The highest metabolism obtained in the control group was plus 3.0 per cent and the lowest −15.0 per cent, the average being −6.0 per cent.

Further investigations are being carried out on a number of racial mixtures and also on pure strains in this region.

CONCLUSIONS

The authors have performed a series of basal metabolism tests and vital capacity measurements on twenty-eight Syrian women. The studies were controlled by observations on seven American, English and German women residents of Syria.

1. Vital capacity values were found to be definitely lower in the Syrian women than in the controls, the difference being 5 per cent.
2. Pulse rates fell within the normal range in both Syrian and control groups.
3. Blood pressure values were within the normal range in both groups.
4. The basal metabolism of the Syrian subjects averages 7.3 per cent lower than the control average. The Syrian average was −13.3 per cent and the control average −6.0 per cent. The Aub and DuBois standards were used.
5. Four subjects of mixed parentage were included in the group. Their average metabolism more nearly approached that of the controls than of the Syrians.
6. The Aub and DuBois tables give a value at least 12 per cent too high for Syrian women.

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SERUM IRON DETERMINATIONS APPLIED TO THE STUDY OF EXPERIMENTAL ANEMIA

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In a previous communication (1) concerning the relative effect of beef liver and iron salts on the rate of hemoglobin regeneration in experimental anemia from hemorrhage, it was concluded that the iron content of the food administered was the sole regulator of hemoglobin production. This conclusion was at that time somewhat at variance with the findings of Whipple and Robscheit-Robbins (2) who had shown that liver and kidney feeding apparently was of specific value in stimulating hemoglobin production. These workers, in one series of experiments, were able to show an added effect of liver or kidney when an iron salt was fed simultaneously. The question of iron starvation was discussed by Whipple and the conclusion reached that "the effect of iron salts may depend on an actual iron shortage or deficiency or salt inbalance," and that little was known about the relation of these factors to the experimental procedures.

In our experiments it was assumed that a relative iron shortage must be present, since during the experiments, relatively large quantities of iron are removed from the animals in the form of hemoglobin, and that the basal diet was deficient in iron. In the work here reported we have subjected that assumption to experimental proof, by the determination of the iron content of the blood serum during the course of the anemia.

METHODS. The methods used for the production of anemia were fully described in the previous paper and in general follow those of the older investigators, Kunkel (4) and Egers (5). They differ from those used by Whipple and Robscheit-Robbins (6) in three respects: only distilled water was given the dogs, milk was used in place of salmon to insure food consumption, and the duration of the experiments was regulated only by the time necessary to show conclusive results.

Six dogs were used and on each, several separate experiments were carried out in succession in order that the individual reactions could be compared in each case. Iron citrate, liver and spleen were fed in various amounts, and the hemoglobin and serum iron levels determined on each specimen of blood drawn.

Hemoglobin was determined by the Osgood and Haskins modification

(7) of the Sahli method. This method was found to check within 2 per cent in our laboratory with the oxygen capacity method and is much less time consuming than the latter.

Serum iron determinations were made by Elvejhem and Hart's modification of the Thompson method (8). In order to avoid the possible error due to hemolysis the serum was precipitated with trichloracetic acid and the iron content determined on the filtrate. We are indebted to Briggs (9) for this suggestion, and in table 1 are given the control proce-

TABLE 1

	PRECIPITATED HEMOLYZED SERUM PER 100 cc.	PRECIPITATED UNHEMOLYZED SERUM	DIFFERENCES
	m.		
Dog I	1.02	1.01	0.01
Dog 7	0.93	0.92	0.01
Patient A	1.7	1.6	0.1
Patient A	1.3	1.4	0.1
Patient A	1.1	1.2	0.1
Second anemia	1.5	1.3	0.2
Dog 1	0.7	0.7	0.0
Dog 1	1.07	1.06	0.01
Dog 1	1.0	0.98	0.02
Dog 2	0.84	0.80	0.04
Dog 2	0.80	0.80	0.0
Dog 3	1.08	1.06	0.02
Dog 3	0.9	0.89	0.01
Dog 4	0.93	0.91	0.02
Dog 4	0.90	0.9	0.0
Dog 4	1.2	1.18	0.02
Dog 5	0.8	0.8	0.0
Dog 5	0.95	0.92	0.03
Dog 5	1.2	1.19	0.01
Dog 5	0.99	0.96	0.03
Dog 5	1.2	1.17	0.03
Dog 7	1.2	1.18	0.02

dures which were used to establish the validity of the method, Barkan (10) having shown that absorption of iron from cells is inappreciable when serum rather than plasma is used. The determinations were made in duplicate on 10 cc. samples of serum, the blood having been centrifuged as soon as possible after withdrawal. No interference with phosphate was encountered, the removal of this as ammonium phosphomolybdate in accordance with the modification of Elvejhem and Hart was quite satisfactory. Porous bottom filtration crucibles were fitted to special test tubes in order to care for small amounts of liquid. Colorimetric readings were made to an accuracy of 0.03 mgm. of iron.

In table 1 are shown results of the analyses when hemolyzed serum was compared with the filtrate from the clear serum of the same animal. The differences are significant in that the use of the filtrate eliminated small errors due to hemolysis.

In table 2 clear sera were compared with the filtrates from the same sera and the results show that no appreciable amount of iron was lost by this procedure.

RESULTS. The charts have been constructed to show the relation of the serum iron level to the hemoglobin percentage, the blocked areas representing the amount in grams of hemoglobin removed. The latter calculation was made by considering an arbitrary standard of 13.8 grams of hemoglobin to be present when the level was 100 per cent.

In dog 1 (fig. 1) an initial serum iron level of 1.7 mgm. fell to 0.8 mgm. in 15 days following severe hemorrhage, during which time 83.4 grams of hemoglobin had been removed. This serum iron level was maintained

TABLE 2

	CLEAR WHOLE SERUM	PRECIPITATED SERUM	DIFFERENCES
Second anemia	1.0	0.93	0.07
Second anemia	1.3	1.1	0.2
Dog blood	0.7	0.7	0.0
Dog blood	1.5	1.5	0.0
Dog blood	0.75	0.75	0.0
Dog blood	1.25	1.25	0.0
Dog blood	1.0	1.0	0.0
Dog blood	0.93	0.92	0.01
Dog blood	0.87	0.87	0.0

while the hemoglobin level continued to fall, and regeneration was minimal. With the addition to the diet of 0.25 gram of ferric citrate daily, the serum iron level rose with a consequent increase in hemoglobin regeneration. This rise took place in spite of the removal of 57.2 grams of hemoglobin. When iron administration was discontinued the serum iron level fell again to the previous low level.

In dog 2 a small amount of liver (70 grams daily) was fed and a similar but less marked rise in serum iron was followed by increased hemoglobin production. In the same dog iron citrate gave a similar response. In dog 3 the serum iron level was depressed to 0.9 mgm. at one period while at another the level was reduced to 0.7 mgm. per 100 cc. during which time hemoglobin regeneration was at a minimum.

Dog 5 was fed large amounts of liver from the beginning of the experiment. The serum iron level was low and irregular but hemoglobin regeneration was rapid. Spleen feeding gave a similar response. In dog 7

iron citrate was fed from the beginning of the experiment, the serum iron was extremely low, the hemoglobin level fairly constant, while regeneration continued at a rapid rate.

It will be seen from the tables that the serum iron and hemoglobin levels are not consistently parallel. The hemoglobin level is dependent upon the amount of blood being withdrawn and the rate of regeneration, while the serum iron may be low during rapid regeneration of hemoglobin as well as when the body iron stores are not replenished by dietary methods. In two of the experiments (dogs 1 and 3) there was a transient rise in serum iron during the initial hemorrhages as if a mobilization of iron from body stores were taking place.

The normal serum iron values for dogs varies between 1.7 and 1.0 mgm. while during iron starvation the levels vary between 0.9 and 0.7 mgm. per 100 cc. of serum.

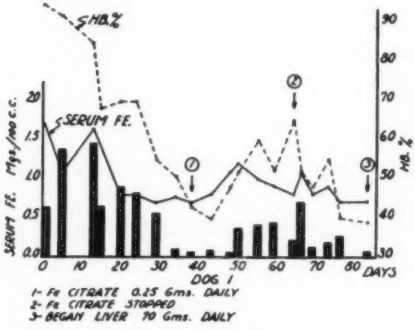

Fig. 1

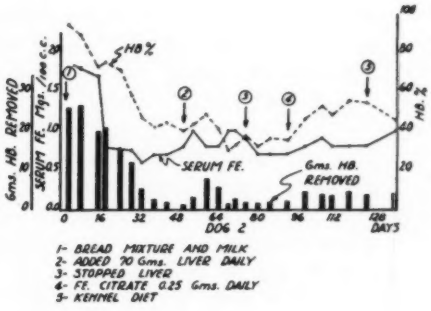

Fig. 2

The clinical application of the relation of serum iron level to hemoglobin regeneration will form a separate report.

COMMENT. With respect to the summation of effect when liver or spleen is fed together with an iron salt, the results of Whipple and Robscheit-Robbins are susceptible of another interpretation. A. C. Curtiss (3) has found in the study of the relation of diet to appetite, that liver extract in a concentration of 2 per cent had a much greater effect upon the appetite of rats and consequently upon caloric ingestion and growth than an equal amount of yeast vitamine powder (Harris). He concludes that liver extract feeding exerts a marked influence on the growth of the animals and although the rôle of artificial diets cannot be disregarded, the results must be considered in the anemia experiments on dogs. It has been shown (11) that liver extract has had no specific effect on hemoglobin production in these animals, but we cannot assume that iron starvation was not present.

It is evident that serum iron determination may be used to estimate the degree of relative iron starvation in experiments of this nature; they may be used also to determine the amount of iron available for hemoglobin production, and as an index of the absorption of iron from the gastro-intestinal tract when iron starvation is present. Clinical studies now in progress show that in secondary anemia the serum iron level may be of value to predict whether or not iron medication is indicated in treatment.

In a separate group of experiments (12), carried out under identical conditions, the microchemical methods of Macallum (13) were used to demonstrate that a decrease in the iron content of the bone marrow cells occurs during the time when the rate of hemoglobin regeneration is at a minimum.

It seems that if a specific stimulus to hemoglobin formation by food products is to be detected by feeding experiments, it must be shown that an abundance of iron is available for the animal, in order to avoid the depressant action of relative iron starvation. The possible effect on hemoglobin production by the stimulating action of the general metabolism by the foods also should receive consideration.

CONCLUSIONS

1. In experimental anemia from hemorrhage iron starvation may be produced with a depression below normal of the serum iron level.

2. In this type of experiment, regeneration of hemoglobin follows the administration of iron, as the citrate or as food iron, with a rise in the serum iron content of the blood.

3. There is no direct relationship between the serum iron level and the hemoglobin level since both are governed by other factors.

4. In this type of anemia iron intake seems to be the sole regulator of hemoglobin production.

Dog 3

DAY NUMBER	HEMOGLOBIN REMOVED	HEMOGLOBIN LEVEL	SERUM IRON	DIET
	grams	*per cent*	*mgm. per 100 cc.*	
1	31.5	122	1.16	Bread and milk
7	19.2	93	2.1	
9	10.9	79	1.3	
14	21.0	80	0.98	
18	11.2	65	0.9	
23	7.3	53	0.87	
28	1.0	42	0.9	
32	1.1	40	0.88	Began Fe Cit. 0.25 gm. daily
37	1.0	41	0.95	
42	1.4	52	1.0	
44	8.8	64	1.2	
49	8.6	61	1.0	
53	10.6	55	0.8	
58	3.5	63	0.8	Discontinued Fe Cit. 0.25 gm.
60	14.0	49	1.08	daily. Kennel diet
62	2.3	41	1.0	
69	2.2	45	0.7	
75	1.7	42	0.7	
82	2.2	40	0.8	
89	6.6	48	0.9	
95	3.5	55	0.95	
107	4.2	61	1.20	
121	3.5	63	1.20	
130	4.1	73	1.30	

Dog 4

DAY NUMBER	HEMOGLOBIN REMOVED	HEMOGLOBIN LEVEL	SERUM FE LEVEL	DIET
	grams	*per cent*	*mgm. per 100 cc.*	
1	41.4	100	1.22	Bread and milk
7	30.6	74	1.1	
10	18.2	66		
17	12.4	60	0.8	
22	10.3	50	0.73	
26	9.0	56	0.6	
31	3.1	55	0.7	
35	1.9	46	0.7	
43	2.5	45	0.8	
				Began raw liver 70 gms. daily
47	1.8	51	0.9	
52	8.86	49	1.01	
57	8.44	51	0.8	
61	6.2	45	1.06	
66	4.3	52	0.08	
				Discontinued liver
69	2.9	42	0.7	
76	1.7	41	0.69	Began Fe Cit. 0.25 gm. daily
83	2.5	45	0.9	
90	5.0	60	0.87	
92	9.9	60	0.93	
97	4.0	58	0.8	
101	4.0	58	0.8	
104	9.5	60	0.9	
				Fe Cit. discontinued 109th day
111	4.2	61	1.0	
121	3.6	66	1.0	Liver 300 gms. daily
126	11.0	54	1.2	
133	15.2	55	1.2	

Dog 5

DAYS	AMOUNT BLED	HEMOGLOBIN REMOVED	HEMOGLOBIN LEVEL	SERUM IRON LEVEL	DIET
	cc.	*grams*	*per cent*	*mgm. per 100 cc.*	
1	150	21.3	103	1.03	Bread mixture
3	200	26.8	97	1.04	
5	86	10.0	91	1.00	
8	190	19.9	76	0.93	
10	100	9.1	66	0.90	
15	40	2.9	52	1.0	
19	25	1.9	55	0.8	19th day 200 gms. liver
24	125	9.1	55	0.95	added daily
29	100	7.7	56	0.8	
33	100	6.9	50	1.05	
37	100	7.9	57	0.91	
39	120	9.1	55		
41	50	3.9	55	1.1	41st day liver discontinued
44	100	7.3	53	0.8	
48	50	3.6	52	0.7	48th day spleen 300 gms.
53	40	2.4	44	0.8	daily
58	40	2.5	45	0.9	
64	60	5.5	66	1.0	
66	116	8.8	58	0.95	
71	50	4.7	68		
75	100	9.6	70	1.2	
78	110	9.8	65	1.0	78th day spleen discon-
82	100	8.4	61	0.99	tinued
85	50	3.2	52	0.95	
94	100	6.9	50	1.2	Kennel diet
99	40	3.1	57	1.1	
109	45	3.4	55	0.9	

Dog 7

DAYS	AMOUNT BLED	HEMOGLOBIN REMOVED	HEMOGLOBIN LEVEL	SERUM IRON LEVEL	DIET
	cc.	*grams*	*per cent*	*mgm. per 100 cc.*	
1	100	14.6	106	1.2	Bread mixture + 0.25 mgm. Fe Cit.
3	110	12.9	85	0.9	
5	120	13.9	84	0.9	
10	100	9.8	71	0.8	Fe Cit. increased to 0.5 mgm. 10th day
12	100	10.2	74	1.1	
15	120	11.3	68	0.9	
19	160	13.8	67	0.8	
23	150	9.9	48	0.8	
27	100	6.6	48	0.7	
31	50	3.0	43	0.7	
38	50	3.7	54	0.7	
45	100	7.6	55		
52	90	5.7	47	0.77	
61	60	3.6	44	0.8	Fe Cit. stopped and began liver on 61st day
64	40	2.4	44	0.9	
68	50	3.5	50	1.1	
73	100	5.5	40	1.2	
80	90	5.0	40	0.93	
87	45	2.8	45	0.80	

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OBSERVATIONS ON THE PREPARATION AND ACTIVITIES OF A VISCERAL ORGANISM

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One of the comparatively recent improvements in physiologic technic is the surgical isolation of the thoracic and abdominal viscera in a living state. Carrel (1913) described a method for removing the thoracic and abdominal viscera en masse from cats and dogs. This visceral organism, as he called the preparation, had to be kept completely immersed in a solution of sodium chloride since otherwise it would survive only a few hours. Death, which eventually ensued in about twelve hours, was ascribed by him to septicemia from portal invasion, to peritonitis and ileus.

To isolate the viscera of an animal from the parietes is a convenient way of studying certain physiologic problems. However, the study of the chemical constituents of the blood of a visceral organism as prepared by Carrel is not satisfactory in view of the fact that there is undoubtedly such extensive diffusion between the blood and the solution of sodium chloride as to make the figures of questionable value.

The visceral organism may be regarded as complementary to the eviscerated preparation. The complete removal of all the abdominal viscera, as practiced by Mann, leaves a preparation that is as physiologic as can well be the case under these conditions. Although the viscera below the diaphragm have been removed and there remain in the animal only the central nervous, muscular and osseous systems, heart, lungs and several endocrine glands, the animal is in good condition with a normal blood pressure and he walks about the room and takes a friendly interest in his environment. It it not likely that this technic can be greatly improved, and it should be regarded as ideally suited for the study of muscle metabolism in general. Since the pancreas has been removed, the preparation must be considered diabetic. This eviscerated preparation is also useful in the study of certain problems regarding the physiology of a dehepatized dog, provided the results are finally confirmed on an animal from which only the liver has been removed without disturbing the other viscera. Since the eviscerated preparation may be regarded as the opposite of a visceral organism, the latter should be of value in a study of the metabolism of the viscera in the

absence of skeletal muscle. By combining the data obtained by means of a visceral organism and the eviscerated preparation, one should obtain data on the functions of the liver that are mutually confirmatory.

It is not our purpose here to review the various excellent methods that have been evolved for perfusing isolated organs mechanically. Such methods have been very useful in the pioneer experiments on the physiology of such structures as the liver, the kidneys and the hind limbs. The intense vasoconstrictor properties of shed blood renders the successful perfusion of an organ difficult, as does also the rapid onset of edema. A distinct step forward in the technic of perfusion was made when Bainbridge and Evans (1914), and later Starling and Verney (1925) perfused the isolated kidney with defibrinated blood by means of a heart-lung preparation instead of a pump. The passage of the blood through the lungs not only aërates it effectively but also removes certain vasoconstrictor substances, which are possibly responsible for the edema. This principle has been used by Priestley (1929) for the perfusion of the hind limb of a dog and also for the perfusion of the liver. However ideal as the heart-lung method of perfusion of an isolated organ may seem for certain studies, it has drawbacks for the perfusion of such an organ as the liver, which is markedly susceptible to asphyxia. The whole question of the action of insulin on the isolated liver must be regarded as unsettled for this reason; during the process of isolating the liver for perfusion, irreparable asphyxial glycogenolysis develops so that one is left in doubt as to the value of any subsequent developments in this organ following the addition of insulin.

We have modified and simplified Carrel's preparation. A visceral organism can be prepared which includes the heart and lungs, the whole gastro-intestinal tract, spleen, pancreas, suprarenal glands, liver, kidneys, ureters, and bladder. The appearance of such a preparation is often macroscopically normal; the liver and spleen have their usual tint; the stomach and gastro-intestinal tract are more active than is the case in an intact animal observed roentgenologically; the intestines absorb food, including fat, as shown by the appearance of chyle in the lacteals: the kidneys secrete urine; the blood pressure varies, and under certain conditions, such as immediately after transfusion, is as high as 90 mm. of mercury.

Our method for the preparation of a visceral organism is quite feasible, and in experienced hands the time taken for the procedure is less than an hour. This technic rarely fails. Cats and small dogs that have been fasting for forty-eight hours are most suitable. In principle the method consists in ligation and section of all the vessels that connect the aorta and vena cava to the parietes. The viscera can then be removed from the body by means of a moist towel improvised into a hammock. It is wise, whenever possible, to leave intact any veins from the parietes until all the arteries have been tied. This measure conserves blood. Toward the

end of the operation it may be considered advisable to give a transfusion of 5 to 10 cc. of blood for each kilogram of body weight. This is best injected by means of an oiled syringe into the external jugular vein. Should this technic be considered inconvenient, a supply of fresh heparinized blood may be kept on hand.

TECHNIC OF THE OPERATION. Through a median-line incision under ether anesthesia, the intestines are retracted to the left and the ileum is severed about 3 cm. from the ileocecal valve. The bowel distal to this is excised after the inferior mesenteric artery and the necessary branches of the superior mesenteric artery and vein have been tied. The aorta below the renal artery is sectioned between ligatures, as is also the vena cava. The renal capsule is freed from the surrounding connective tissue. The ureters are ligated and sectioned. The aorta and vena cava are retracted upward, exposing the lumbar arteries and veins, which are cut and tied. Usually three pairs are encountered by the time the operator has reached the pillars of the diaphragm. The phrenico-abdominal artery and vein are similarly tied near their origin on each side. The pillars of the diaphragm are now detached from the vertebrae. The abdomen is closed with clamps and the thorax is entered by an incision which is strictly in the middle of the sternum. The open chest is strongly retracted. The internal mammary arteries are tied at their origin. The pericardial attachment to the diaphragm is severed and the central tendon of the diaphragm is trimmed around the caval orifice. The diaphragm is then cut as close as possible to the esophagus and aorta until it is completely separated from these structures. An attempt is not made to preserve the thoracic duct. Several small bleeding vessels are encountered during the separation of the diaphragm, and these must be ligated. This method of opening the thorax, followed by the procedures on the diaphragm, results in excellent exposure of the intercostal vessels in the thorax. The intercostal arteries on the left side are tied by means of an aneurism needle close to their origin from the aorta. In small dogs they can be tied two at a time, and in cats three at a time. The exposure should be such as to enable the operator to visualize clearly every intercostal vessel. It is occasionally difficult to see the origin of the lower two intercostal vessels, in which case a mass ligature is tied in that region of the aorta. The parietal pleura is rapidly stripped from the aorta on the right side, with exposure of the intercostal arteries, and this process is repeated for the intercostal vessels on the right side. If feasible, the azygos vein is not sectioned until the intercostal arteries have been tied. In most cases it will be found more convenient to cut and tie the azygos vein in order to get the proper exposure of the upper intercostal vessels. The brachiocephalic and left common carotid arteries are tied and cut. At this stage of the operation one can easily determine the

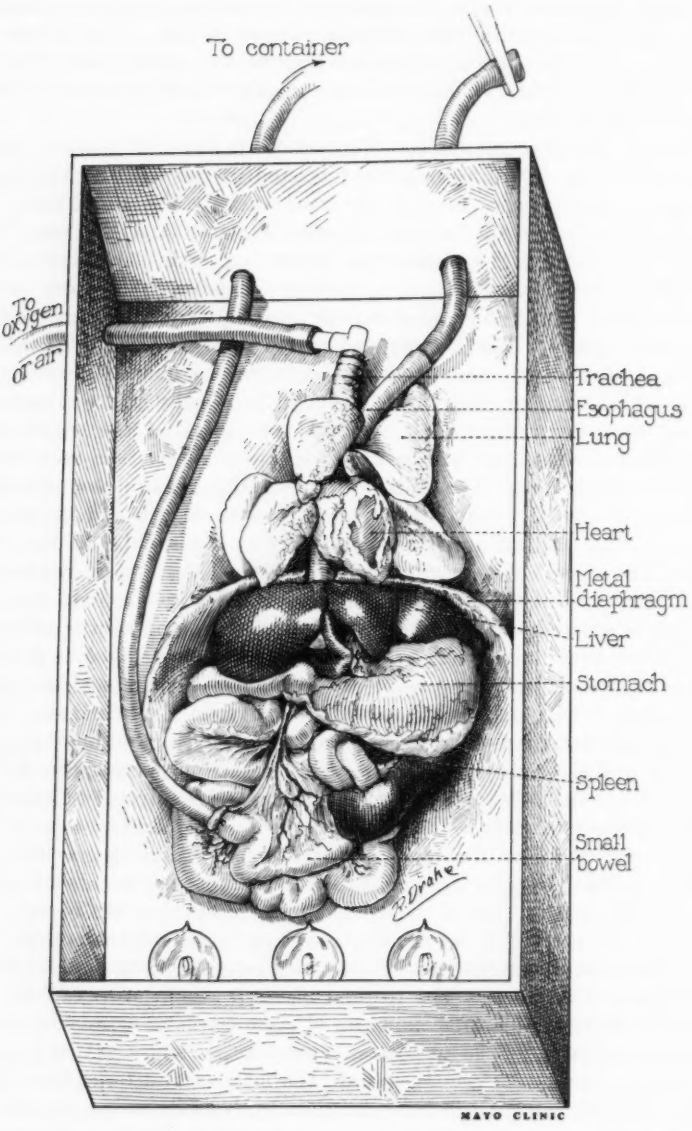

Fig. 1. Arrangement of visceral organism. The box is placed on top of a Brodie table, and is covered with plate glass.

condition of the blood pressure by palpating the aorta. If the blood pressure is low, transfusion by the jugular vein should be given. Occasionally the blood pressure is higher than normal, in which case the transfusion should not be given, since this leads to congestion of the viscera and predisposes to rapid edema. The superior vena cava is then tied and cut on the cardiac side of its last tributary.

Everything is then ready for the removal of the viscera. The intercostal vessels are cut on each side distal to the ligature and any adherent strands of tissue are rapidly snipped. The esophagus is clamped, the trachea is liberated rapidly, and the visceral organism is then placed on a warm towel which serves as a hammock. It is carefully examined for the slightest trace of oozing, and when absolute hemostasis has been accomplished, it is transferred to a warm box or incubator. The intestines are inclined for at least 12 cm. above the right auricle to facilitate venous return. Provision is made by means of towels or a suitable metal diaphragm to prevent occlusion or kinking of the inferior vena cava by the liver (fig. 1). By means of a thread attached to the pericardium the heart is prevented from compressing the great veins.

Just previous to the establishment of pneumothorax, a cannula is inserted into the trachea and artificial respiration is maintained by means of air or oxygen passed over an ether bottle. It requires care on the part of the anesthetist to provide adequate ventilation during the time that the lungs are being compressed for ligation of the intercostal vessels. As soon as the brachiocephalic and left brachial arteries are ligated the anesthetic is discontinued.

It is possible to proceed rapidly with the operation of preparing a visceral organism without doing a transfusion until the preparation has been completely isolated and placed in the incubator. If the heart has stopped beating a transfusion into the brachiocephalic artery will almost invariably revive the preparation immediately. When the heart is beating but feebly a slow intravenous transfusion will usually restore the blood pressure approximately to normal. Occasionally an intravenous transfusion when the heart is beating feebly will cause marked dilatation of the heart, in which case one must rapidly remove the injected blood and transfuse intra-arterially. This practice is in accord with the known principle that the prime essential in resuscitating a dead or dying heart is to provide suitable intracoronary pressure. Although it is feasible to complete the operation without transfusion, we do not recommend this procedure. The cause for cardiac failure during the operation is undoubtedly a coronary pressure that is too low for the requirements of the heart. This low blood pressure is the result of removal of the vasomotor centers, of the unavoidable loss of blood into the parietes, and of the more or less avoidable cooling of the tissues by the great exposure.

To keep the visceral organism warm is a simple matter if the insufflated air is not allowed to escape in the incubator, for which purpose the orifice in the cannula is closed and an incision is made outside the incubator in the tubing leading to the cannula. It is wise to pass the air through a large bottle of hot water.

Usually it is advisable to transfuse a visceral organism as soon as it is placed in the incubator. The amount of blood given this time should be enough to raise the blood pressure to about 80 mm. of mercury. This is usually about 4 cc. of blood for each kilogram of the animal's original weight. This figure varies for the size of the intestines. Certain small dogs have very large intestines, in which case more blood will be required to meet the capacity of the organism. After this has been done a drainage tube is tied into the ileum and led to the outside through a hole in the incubator. The esophagus is similarly treated. Unless there is some experimental objection to it, 25 cc. of warm Locke's solution is injected through the stomach tube into the stomach. This fluid is slowly absorbed and serves to stabilize the blood pressure; at the same time it works its way down the ileum rapidly and appears externally, being tinted with bile and containing some ileal contents. It probably is useful in postponing the inevitable distention and autodigestion of the intestines by draining the septic contents to the outside.

It is absolutely essential to elevate the posterior portion of this preparation to facilitate the venous return to the heart, otherwise frequent transfusions will be necessary to maintain physiologic blood pressure and there will be pronounced edema and congestion of the viscera. Usually we have inclined the intestines about 15 cm. above the heart by placing a block of wood at one end of the support. This procedure serves also to increase the intracoronary pressure by the hydrostatic effect of the heart pumping blood against the force of gravity.

Behavior of the visceral organism. The heart rate is about 120 beats each minute at body temperature and occasionally increases to 200 or more. These increases are more likely to occur in conditions of low blood pressure or following poor ventilation, and they may be the result of a discharge of epinephrine from the medulla of the suprarenal glands.

The lungs need not become edematous even when the preparation has been isolated for as long as twelve hours. This may be contrasted with the invariable onset of rapid edema of the lungs which ensues in a heart-lung preparation. Since in our experience pulmonary edema is just as constant in a heart-lung-liver perfusion and in a heart-lung-leg perfusion, we are not inclined to ascribe the edema to the absence of a capillary hormone, especially in view of our observations that edema need not occur in the lungs of the visceral organism. Nor have we found any evidence that the type of artificial respiration is essentially the factor in producing pulmonary

edema. We are inclined to ascribe the pulmonary edema of a heart-lung preparation to the development of a capillary poison in the blood in consequence of some change it undergoes when in contact with a non-endothelial surface.

The intestines present a remarkable picture in a visceral organism. The transition from a period of quiescence during the intra-abdominal manipulations at the beginning of the operation to a condition of intense activity when the organism is placed in the incubator, is a striking demonstration of the inhibitory effect of the central nervous system on movements of the intestine. The stomach and intestines of a visceral organism for the first few hours are in a veritable turmoil of activity. After the introduction of 25 cc. of Locke's solution into the stomach, strong peristaltic waves beginning about the middle of this organ sweep rapidly and concertedly to the pylorus, which admits large gushes into the duodenum. The first portion of the duodenum shows frequent powerful rhythmic contractions and one can readily comprehend from the constantly active appearance of this portion of the duodenum why ulcers are so prone to develop in this situation. We have emphasized this point regarding the etiology of peptic ulcer. Pendular movements are present along the whole length of the small intestine and waves of peristalsis (usually showing inhibition in advance of the wave of constriction) can be seen regularly. On one occasion we observed peristaltic rush. Alvarez' observation that the upper part of the bowel is more rhythmic than the lower part can be easily verified in the visceral organism.

There are today many methods for perfusing strips of bowel of greater or less length. Although undoubtedly these methods have their use in physiology we wish to propose the visceral organism as the preparation best suited to the study of the concerted activity of the denervated bowel. In the past all the methods for studying the isolated intestine have omitted the stomach. In a visceral organism the stomach is probably the most rhythmically active in the whole tract.

It is advantageous in preparing a visceral organism to include the kidneys even if the ureters are ligated. The circulation through the renal arteries ensures peripheral resistance and a venous return in case of temporary obstruction and accidents to the portal circulation. Beyond the fact that the kidneys secrete urine, we have not had experience with the behavior of the kidneys in such a preparation. With the removal of the preparation from the antidiuretic influence of the pituitary gland, there tends to develop a condition of ingravescent polyuria which rapidly depletes the volume of blood.

The spleen is normal in color and usually is enlarged. The function of the spleen in regulating the blood volume of an intact animal is apparently dependent on the central nervous system. Even when the blood

pressure is so low that there are no pulsations in the mesenteric arterioles, the spleen does not shrink. At this point manual compression of the spleen causes it to shrink and the effect of extruding the contained blood on the blood pressure can be easily demonstrated by watching the small mesenteric arteries. When these have stopped pulsating as a consequence of the low volume of blood, compression of the spleen for a few seconds almost immediately causes pulsations to reappear in the arterioles. These pulsations persist for several minutes after the compression of the spleen has ceased, and slowly disappear as the spleen regains its former size.

The liver in a good visceral organism is normal in appearance. One of the surest signs that something is amiss with the preparation is a change in the color of the liver from an arterial pink to a venous blue-black. This change is usually the result of oligemia so that the blood becomes excessively reduced as it passes through the liver. In some cases this color is the result of the use of too much blood in the transfusion, so that the blood becomes crowded into the liver. The liver of the dog is much more vulnerable than the liver of the cat to the injuring influence of an abnormal volume of blood.

The pancreas appears healthy and apparently does not undergo autodigestion, at least during the early part of the life of a visceral organism.

Two properties of a visceral organism unfortunately limit its usefulness for prolonged experiments: the constant appearance of more or less edema of all the abdominal viscera, and, probably dependent on the foregoing, the need for repeated transfusions of blood or plasma. If the loss of blood volume is very great on account of edema, the loss has to be rectified by frequent transfusions. A little consideration will show that these disturbances are inevitable, since the lymphatic drainage from the viscera has been completely obstructed; the lymph nodes along the route of the mesentery become more or less engorged, sometimes to the extent of embarrassing the circulation through the portal vein. In some preparations the lymph flow is apparently not very great; at any rate, edema is much less, especially in the case of cats, in which animal transfusions need not be given so often. We have seen a cat visceral organism survive for more than four hours without a transfusion. On the average, transfusions of greater or less volume have to be given about every two hours. The indication for a transfusion is marked enfeeblement of pulsations in the mesenteric arterioles. The quantity of blood or plasma transfused depends on the blood pressure. When this is so low that pulsations cannot be seen, 4 cc. of blood for each kilogram of the animal's original weight should be administered into the superior vena cava. If the experiment is of such a nature as to interdict the injection of blood containing an anticoagulant, the oiled syringe technic is employed for transfusion of fresh blood.

We can state with confidence that the cause of death in such a prepara-

tion is nearly always cardiac. When the organism is carefully watched and transfused as soon as the pulsations in the mesenteric arterioles become weakened, the visceral organism lives in good condition for more than twelve hours.

After about five hours the intestines become distended and their activity is somewhat impeded, especially in the dog. Their appearance is such as to suggest cyanosis, although the liver and spleen seem healthy. The explanation for this appearance is manifest at necropsy. This is the result of marked erosion and autodigestion of the whole gastro-intestinal tract. It appears that as soon as the intestine is isolated from the body in this manner it is no longer able to protect itself against digestion by its own enzymes and against bacterial invasion. We recall in this connection the observation that acute peptic ulcers develop after section of the splanchnic nerves.

SUMMARY

A technic is described for isolating in a living condition the thoracic and abdominal viscera of cats and dogs. The behavior of such a visceral organism is briefly described.

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THE RELATION BETWEEN STATURE AND THE LATENT PERIOD OF THE KNEE JERK

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We have begun the study of the latent period of the knee jerk in the hope of finding therein a sensitive indicator of the functional condition of a portion of the central nervous system, which might be useful in the laboratory investigation of mental disorders.

The recent reports of Travis (1928) and Travis and Hunter (1928) that this time measurement, which they call "knee jerk reflex time," correlates inversely with intelligence, stimulated our efforts. Our observations contradict this proposition. (Whitehorn, Lundholm and Gardner, 1930.) We have, however, reached one conclusion of physiological interest which we wish to report at this time, namely, that the latent period of the knee jerk is determined in large part by the stature of the subject, tall subjects having a longer latent period than short subjects.

METHOD. The term "latent period of the knee jerk" is used here to signify the time between the first impact of a mechanical tap on the ligamentum patellae and the beginning of the electrical disturbance in the extensor muscles, initiating their response. For recording and measuring this time interval we used a Hindle electrocardiograph no. 2, obtained from the Cambridge Instrument Company, Ossining-on-Hudson, New York. One of the electrodes (strips of "German silver" about 1 cm. wide) was put on the front of the thigh, held by rubber bands over a small piece of absorbent cotton, wet with 15 per cent salt solution. The other electrode was similarly applied to the calf of the leg. The string (gilded quartz, 3500 ohm) was adjusted to such tension that 6 m. v. produced 1 cm. excursion of the shadow at 600 diameters magnification.

To signalize the moment of stimulation we used a potentiometric arrangement to apply 0.5 millivolt to the galvanometer string. The signal terminals were short circuited through a special key (represented in fig. 1) attached to a wooden bar, held firmly against the ligamentum patellae. A sharp blow upon this bar, constituting the stimulus for the knee jerk, opens the key, thereby signaling the moment of stimulation. This signal device has been checked by striking it while clamped in position to throw its shadow on the photographic record. The movement of the bar

and the movement of the string begin simultaneously so far as such observations could determine.

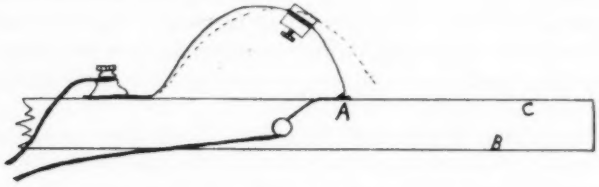

Fig. 1. The edge of the wooden bar at *B* is held firmly against the ligamentum patellae while the bar is stuck at *C* with a small wooden hammer. The weighted metal strip is thereby released from contact with screw head *A*, and springs out to the dotted line position, so that it cannot touch *A* on the rebound.

The bromide paper in the camera we ran at a speed of about 200 mm. per second.

To measure the distance of about 4 mm. representing the latent period we used a special glass scale on which were etched two fine straight lines forming a v (at a slope of 1:5), graduated in millimeters along one line. By proper manipulation of this scale, placed directly upon the photographic record, one can readily estimate distances under reading glass magnification to 0.05 mm., which means 0.25 sigma. The practical limit of precision is set by the uncertainty of judging the precise beginning of the response deflection. This uncertainty varies with sharpness of deflection and of photography. To keep this judgment error as nearly constant as possible, all records have been measured by the same person.

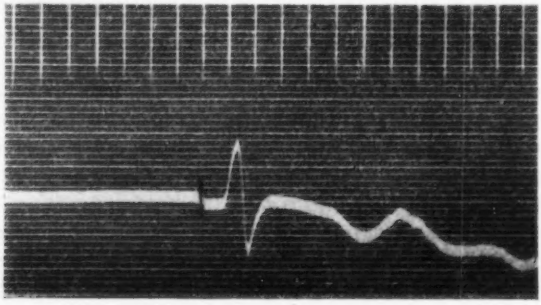

Fig. 2. Galvanometer record of a lively knee jerk, latent period 0.0204 second

These time measurements were corrected, of course, for the fluctuations in speed of film. Because of a slight irregularity in the 0.020 second intervals of the synchronous motor time-marker, we estimated this cor-

rection factor from half-second intervals. (A fly-wheel on the camera insures fairly uniform speed over brief periods.)

PROCEDURE. The subject lay on his back in bed, with the lower legs hanging from the knees over the end of the bed. The electrodes were placed in position. One operator then manipulated the recording devices while the other applied stimuli, five on one leg, then, after shifting electrodes, five on the other.

At the beginning of our study it was uncertain how much depended upon intensity or regularity of stimulus or upon precise position of knee and leg or other parts of the body (e.g., "reënforcement"). We therefore compared on the same subject, numerous crude variations in force of tap, and posture of subject. We also put varying thicknesses of soft rubber between stimulus bar and tendon, and varied the force with which this bar

TABLE 1

Different positions of thigh electrode

	POSITION DESIGNATED					
	A	B	C usual position	D	E	F
Distance from C (cm. proximal or distal)	10 prox.	2.5 prox.	0	5 distal	10.5 distal	15 distal
Latent period (sigma, av.)	19.42	20.23	20.47	21.32	24.10	26.10
Velocity, estimated (mm. per sigma)	93	104	59	20	23	
	Average, upper thigh 85			Average, lower thigh 21		

was held against the tendon. Without attempting a complete and precise analysis of our crude experiments we felt assured of the relative unimportance of these factors. Consequently we judged it unnecessary, at least for our present study, to construct special machinery for controlling the posture of the subject or for tapping the tendon.

As for the comparison of right leg with left leg, our experience indicates the absence of any considerable systematic difference. In 57 normal subjects, the latent period for the right leg averaged 21.46 sigma, for the left leg, 21.40.

The latent period measurement varies with electrode position. The conception that the knee jerk is a reflex implies, if one assumes a constant rate of nerve conduction, that the latent period should depend upon the length of the pathway involved. This implication has been definitely

expressed and considered by Snyder (1910), Hoffmann (1910) and other investigators. During our preliminary experiments, we have observed, as did Hoffmann (1910), that the latent period could be varied by changing the position of the electrode on the thigh—proximal positions giving shorter latent periods than distal positions. Our experiments on one individual are summarized in table 1.

From the point of view of practical technique these results indicate the need for standardizing the electrode position, as a prerequisite for the comparison of one person with another. We have followed Travis' practice of placing the electrode midway between the anterior superior spine of the ilium and the upper border of the patella.

Parenthetically, these observations provide some evidence on the old controversy, whether the knee jerk is a true reflex or a direct response. In the latter case, one would most simply assume that the electrical disturbance would start near the site of stimulation and travel upward. Our contrary finding is obviously in closer harmony with the reflex theory.

Our estimates of the velocity with which this electrical disturbance travels down the thigh are not very exact, but, even so, demonstrate the marked difference between upper and lower thigh. Proximally, the rate is within the range of mammalian nerve conduction rate (see below); distally it slows down almost to the rate of conduction in mammalian muscle (Matthias, 1893). Perhaps in the lower part, the excitatory process does have to travel along muscle fibers, but in the upper part can make better speed along the branches of the femoral nerve supplying the different parts of the quadriceps femoris muscle group. We have not attempted a further study of this velocity variation.

The relation between stature and the latent period of the knee jerk. The variation of the latent period measurements with different electrode positions strongly suggested that the height of the subject might be of some importance, although Travis and Hunter have (1928) stated that "Reflex time is seemingly independent of the height or size of the subject."

We measured the latent period in three small boys and found it much shorter than in adults. An age factor, if such there be, might, however, obscure the interpretation of this comparison. The importance of height is clearly demonstrated, however, in a series of 57 normal adults (38 men and 19 women), varying in height from 58 to 73 inches, and in age from 18 to 59 years. These subjects were nurses and physicians at this hospital. Table 2 presents our measurements on the latent period of these subjects (and the three boys), together with other data possibly related thereto. Under "latent period" are given for each individual the mean of the measurements on both legs (usually ten, five for each leg, sometimes more, rarely less) and the standard deviation of these measurements.

The most striking correlation found in this table is between height and latent period, for whose clearer exhibition we constructed figure 3. The coefficient of correlation (using Pearson's formula) is, for the fifty-seven adults, 0.761 ± 0.038. (Inclusion of the three boys would raise it to 0.858 ± 0.023, but it seems to us preferable to exclude them from statistical consideration, as their measurements fall in a range insufficiently explored.)

In general a 20 sigma latent period of the knee jerk corresponds to a height of five feet and two inches. The taller subjects have longer latent periods, probably because their reflex arcs are longer. The effect of varia-

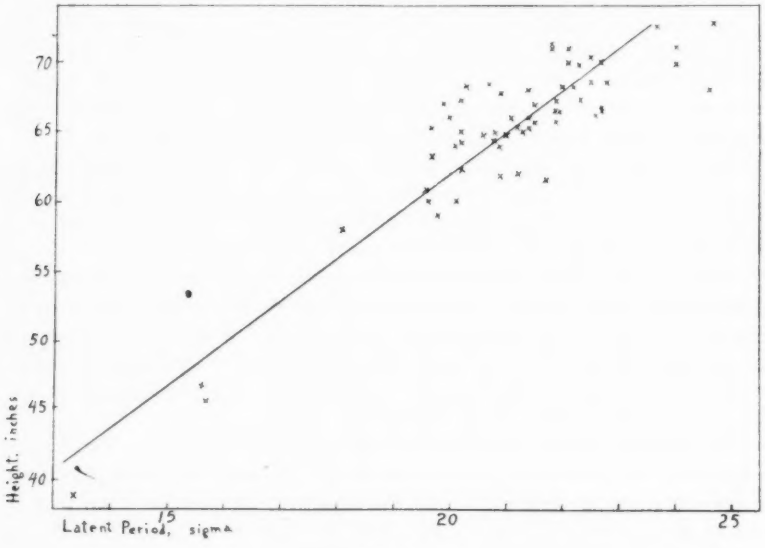

Fig. 3

tions in height may be computed roughly by allowing one sigma for every three inches of height.[1]

Although it is difficult to assign a definite neurological significance to

[1] Calculated from the coefficient of correlation (0.76), the standard deviations (1.38 sigma and 3.55 inches) and the means (21.43 sigma and 66.29 inches), the appropriate regression line indicates:

$$\text{Latent period (in sigma)} = 1.8 + 0.296 \times \text{Height (in inches)}.$$

This formula of course includes a long exterpolation from the range of our observations to the base line, and implies a strictly linear relationship which probably would not hold true in the lower height ranges. It does serve however to indicate that there is a small portion of the latent period independent of height influence.

this particular numerical ratio, a few calculations are suggestive. If a three inch increase in height added three inches (75 mm.) to the length of the reflex arc, the rate of conduction would appear to be 75 mm. per sigma, which is considerably slower than Piper's (1908) figures for motor nerve conduction rate (120 meters per second) but which corresponds with Beritoff's (1929) figure (70–80 meters per second) for reflex arcs in cats.

The application of this estimated conduction rate to the whole length of this reflex arc gives an interesting result. We have made a few estimates of the length of the arc by measuring from the patella to the eleventh thoracic vertebra and back to the mid-thigh (assuming the synapse to be in the lumbar cord). The reflex arc, so measured, has a length about four-fifths as great as the height, for example, 55 inches (1400 mm.) in a subject whose height was 68.3 inches (latent period 22 sigma). At a rate of 75 mm. per sigma, conduction over this arc would require about 18.5 sigma, leaving the remainder of the latent period, 3.5 sigma, for latencies of afferent nerve ending, synapse and motor nerve ending.

The numerical value of this remainder is largely affected by the conduction rate assumed. Estimates of much greater validity have been made by Jolly (1911) for the knee jerk in spinal cats, where the conduction time, because of the short nerves, is relatively less important. Assuming a conduction rate of 120 meters per second, he calculated for latency of motor nerve ending 2.38 sigma, and for synapse time 1.8 sigma. The latency of afferent ending he found negligible, if the afferent path starts in the tendon, as we have assumed in our measurements. The sum of these items (4.18 sigma) exceeds our estimate, in man, by 0.7 sigma. To match it exactly would require an average conduction rate of 79 meters per second. In the absence of direct evidence that the synapse time in man is considerably greater than that in cats, we therefore infer from these calculations that the knee jerk reflex arc in man has a conduction rate approximately 75 to 80 meters per second.

Other possible influences on latent period. A careful examination of our data has not disclosed any factor so important as height. The only other item in our data which has a comparable statistical importance is weight. It is true that the coefficient of correlation between latent period and weight has a fairly high value, 0.53 ± 0.065, but it is distinctly less than that for height, and the statistical relationship is rationally explicable as a mathematical effect of the height-to-weight correlation.

In order to make sure whether or not there was a weight influence independent of the height (rationally conceivable, for example, as an influence of obesity on conduction time or on synapse time), we have calculated the correlation of weight with the figure which represents the difference between the latent period observed and the latent period to be expected for the subject's height. Judged by this criterion, there is no significant influence of weight, independent of height.

TABLE 2

SUBJECT			LATENT PERIOD		HEIGHT	WEIGHT	AGE	DEVIATION FROM HEIGHT RULE		
Sex	Number	Remarks	Mean	S.D.				Calculated from height	+	−
			σ	σ	*inches*	*pounds*	*years*			
M	1		24.7	0.28	72.8	200	25	23.6	1.1	
M	2		24.6	0.78	68	158	30	22.0	2.6	
M	3		24.0	0.58	71	143	26	23.0	1.0	
M	4	Very lively	24.0	0.44	69.8	157	24	22.6	1.4	
M	5		23.7	0.49	72.5	205	38	23.5	0.2	
M	6	Very lively	22.8	0.66	68.5	154	21	22.2	0.6	
M	7		22.7	0.28	66.8	174	55	21.6	1.1	
M	8		22.7	0.26	66.5	150	59	21.5	1.2	
M	9		22.7	0.48	70	189	43	22.7	0	
M	10		22.6	0.32	66.3	149	24	21.4	1.2	
M	11	Very lively	22.5	0.34	70.3	162	26	22.8		0.3
M	12		22.5	0.55	68.5	159	24	22.2	0.3	
M	13		22.3	0.33	67.3	133	22	21.8	0.5	
M	14		22.3	0.41	69.8	143	24	22.6		0.3
M	15		22.2	0.72	68.3	164	28	22.1	0.1	
M	16	Very lively	22.1	0.49	71	254	18	23.0		0.9
M	17		22.1	0.76	70	196	45	22.7		0.6
M	18		22.0	0.31	68.3	162	42	22.1		0.1
M	19	Very lively	21.9	0.32	66.5	152	22	21.5	0.4	
M	20		21.9	0.31	67.3	149	20	21.8	0.1	
F	1		21.9	0.47	66.5	119	22	21.5	0.4	
F	2	Lively	21.9	0.49	65.8	118	21	21.3	0.6	
M	21	Very lively	21.8	0.92	71.3	164	24	23.1		1.3
M	22		21.8	0.52	70	144	24	22.7		0.9
F	3		21.7	0.19	61.5	120	24	19.8	1.9	
M	23		21.5	0.24	67.3	177	26	21.8		0.3
M	24		21.5	0.59	65.7	152	21	21.2	0.3	
M	25		21.4	0.68	68	174	22	22.0		0.6
M	26	Lively	21.4	0.54	66	140	19	21.3	0.1	
M	27		21.4	0.77	65.3	137	43	21.1	0.3	
M	28	Lively	21.3	0.35	65	164	45	21.0	0.3	
F	4		21.2	0.38	65.3	150	21	21.1	0.1	
F	5		21.2	0.70	62	147	28	20.0	1.2	
M	29	Lively	21.1	0.53	66	134	21	21.3		0.2
F	6	Very lively	21.0	0.45	64.8	118	21	20.9	0.1	
M	30		20.9	0.40	64	139	23	20.7	0.2	
F	7		20.9	0.58	67.8	162	31	21.9		1.0
F	8		20.9	0.48	61.8	122	21	19.9	1.0	
F	9	Very lively	20.8	0.18	64.3	129	41	20.8	0	
F	10		20.8	0.41	63	150	27	20.3	0.5	
F	11		20.7	0.42	64.8	110	19	20.9		0.2
M	31	Lively	20.6	0.36	68.3	131	29	22.1		1.5
M	32	Very lively	20.4	0.35	67.3	133	26	21.8		1.4

TABLE 2—*Concluded*

SUBJECT			LATENT PERIOD		HEIGHT	WEIGHT	AGE	DEVIATION FROM HEIGHT RULE		
Sex	Number	Remarks	Mean	S.D.				Calculated from height	+	−
			σ	σ	*inches*	*pounds*	*years*			
F	12	Lively	20.4	0.26	62.3	162	25	20.1	0.3	
M	33	Very lively	20.2	0.22	64.3	132	22	20.8		0.6
M	34	Lively	20.2	0.61	65	126	18	21.0		0.8
F	13	Very lively	20.1	0.36	64	118	40	20.7		0.6
F	14		20.1	0.41	60.7	115	27	19.6	0.5	
M	35		20.0	0.32	66	115	28	21.3		1.3
M	36		19.9	0.17	66.5	135	21	21.5		1.6
F	15		19.8	0.33	59	111	19	19.0	0.8	
M	37		19.7	0.17	63.3	157	44	20.4		0.7
F	16		19.7	0.70	65.3	132	23	21.1		1.4
F	17		19.6	0.32	60.8	103	23	19.6	0	
F	18	Very lively	19.6	0.21	60	143	26	19.3	0.3	
M	38		18.7	0.36	61.8	137	21	19.9		1.2
F	19		18.1	0.24	58	95	22	18.7		0.6
Boy	1		15.7	0.68	45.8	48	$5\frac{9}{12}$	14.6	1.1	
Boy	2		15.6	0.41	46.9	45	$6\frac{2}{12}$	15.0	0.6	
Boy	3		13.4	0.72	39	34	$3\frac{9}{12}$	12.3	1.1	

This same criterion (observed latent period)$-\left(\text{calculated latent period, i.e., } 20 + \frac{\text{Height} - 62 \text{ inches}}{3}\right)$, we have also employed in seeking to detect other possible influences. The figures representing this difference are given in the last columns of table 2.

With regard to sex, the women appear to have latent periods very slightly higher, on the average (0.24 sigma), than those of men, after making the allowance for height. Possibly this slight and questionable difference might be explained by reference to the sitting height, as this measurement is relatively greater in women than in men (Galton, 1889). Unwisely we neglected to measure the sitting height of our subjects.

So far as our observations carry us, age in itself has no effect on the latent period of the knee jerk, if allowance is made for the influence of height. Our data on age, however, are not fully satisfactory for the frequency distribution is quite abnormal, being overloaded at the 20 to 25 year range and sparse elsewhere.

While performing our experiments, we made special note of those persons who had lively or very lively knee jerks, as recorded under "remarks," in table 2. Neither of the groups so designated have latent periods significantly less than would be expected from the subjects'

heights. The slight statistical difference (0.11 sigma) is even less than what we had expected from a purely technical cause, namely, the greater ease of locating on the photographic records of the lively knee jerks the exact beginning of the electrical disturbance, for these are the records which typically show the sharpest and widest deflections of the galvanometer string.

SUMMARY

The latent period of the knee jerk, measured as here described, is relatively constant for individual persons, as compared with the differences between persons. These differences are attributable largely but not wholly to differences in height, presumably because the length of the reflex arc depends upon height. Tall persons have a longer latent period than short persons. The conduction rate in this reflex arc appears to be about 75 to 80 meters per second.

Our observations reveal no influence on this latent period by other factors studied, including weight, sex, posture, age ("mental" or chronological), intensity of stimulus and the apparent liveliness of response.

Acknowledgment. We wish to express our hearty thanks to those who have served as subjects in these experiments, and especially to Mr. George E. Gardner for assistance in the laboratory.

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STUDIES OF VENTRICULAR FIBRILLATION CAUSED BY ELECTRIC SHOCK

I. The Revival of the Heart from Ventricular Fibrillation by Successive Use of Potassium and Calcium Salts[1]

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It may be accepted as established that cardiac death produced by electric currents is due to ventricular fibrillation. But scientific literature dealing with ventricular fibrillation induced in various ways records many instances in which revival is supposed to have occurred spontaneously or appears to have been effected by a variety of procedures.[2] As far as the dogs' or human hearts are concerned, a careful evaluation of the experimental evidence places each reported instance of recovery into one of several classes, viz., *a*, instances in which spontaneous recovery occurred under conditions unattainable in the intact heart; *b*, instances in which recovery resulted from use of methods not applicable to the intact heart, and *c*, instances of supposed spontaneous recovery in which it was not demonstrated that the condition was identical with fibrillation produced by electric currents.

In the latter class fall many reported cases of revival from electrocution, for generally it is not demonstrated by direct observations or by electrocardiographic records whether the ventricles have merely stopped or are in a state of active fibrillation. This is of fundamental importance in judging the efficiency of any measure, for, if the normal ventricles of larger animals are actually thrown into fibrillation by faradic stimulation, a coördinated beat cannot possibly be restored until every vestige of fibrillary activity has disappeared. We have demonstrated this to our complete satisfaction as far as the dog's heart is concerned. In going over our experimental notes of the last 10 years, we find observations on fibrillation

[1] Published by The Physiology Committee on Electric Shock.

[2] For reviews covering this portion of the literature, cf. de Boer, Ergenbnisse der Physiol., 1923, xxi, 32; Garrey, Physiol. Reviews, 1924, iv, 218; Dock, Amer. Heart Journ., 1929, iv, 709.

in 208 dogs. Not a single instance of spontaneous recovery occurred. We offer this as statistical evidence of our assertion that spontaneous recovery does not occur in the normal dog's heart. This does not preclude the possibility that the hearts of larger animals may be sensitized in such a way that a state grossly resembling but not actually identical with fibrillation can develop after electric shock, from which recovery may be possible.

In the treatment of fibrillation it is therefore necessary either to wait until the incoördination has naturally run its course or to use some agent known to abolish the fibrillary process.

THE IMPORTANCE OF ARTIFICIAL INHIBITION OF FIBRILLATION. Before we assume the need of abrogating fibrillation artificially, it is important to establish the natural duration of the process in the dog's heart. This can be done by noting the time elapsing until no evidence of fibrillary movement is discernible when the heart is inspected under good illumination. It is preferable, however, to watch an electrocardiograph string and wait until movement no longer occurs. In comparing the two methods we frequently found that electrical oscillations persist 2 to 8 minutes after all visible evidence seems to have disappeared. We have studied the duration of the fibrillary process in many hearts by the latter method. The results of 29 experiments may be summarized as follows:

Cessation within	7.5 to 10 minutes	—2 instances
Cessation within	11 to 15 minutes	—4 instances
Cessation within	16 to 20 minutes	—3 instances
Cessation within	21 to 30 minutes	—13 instances
Cessation within	31 to 50 minutes	—7 instances

It is obvious that fibrillation as a rule lasts from 15 to 50 minutes, the average duration being 24 minutes.

There is no doubt that revival of the heart itself is possible after longer intervals of time. But the hope of reviving the animal as a whole is not only conditioned by the viability of the heart but also by the time that the central nervous system can withstand the anemia resulting from complete stoppage of the circulation. The results of Stewart, Guthrie, Burns and Pike (1906) have conclusively demonstrated that $7\frac{1}{2}$ minutes is the average interval of complete anemia tolerated by the central nervous system and that this is rarely extended to 15 minutes. Further observations are therefore really unnecessary, but we may state that in experiments in which anemia of the central nervous system was produced by fibrillation rather than by ligation of cerebral vessels, this interval was certainly not extended by more than a very few minutes. The necessity for employing some agent to reduce the natural period of fibrillation becomes apparent.

POTASSIUM INHIBITION. It was only natural that the use of potassium salts to check ventricular fibrillation and the subsequent employment of

calcium salts to restore coördinated vigorous beats should have suggested itself to laboratory investigators. It has long been known (1903) that an excess of potassium ions in the perfusion fluid constitutes the method *par excellence* for stopping a perfused heart, regardless of whether it is beating regularly or fibrillating. By then washing out the excess of potassium with normal Locke's or Tyrode solution, or by neutralizing its action with a slight excess of calcium, a normal beat is easily restored.

Since the circulation ceases promptly when the ventricles fibrillate, the choice of a procedure for applying these agents effectively to the heart *in situ* becomes a matter of considerable importance. The establishment of dosages which on the one hand are effective and on the other are not toxic also becomes a problem for careful investigation. Four possible methods of administration suggest themselves:

a, Intravenous injections of fluid sufficient in volume to enable substances to reach the heart; b, intra-arterial injections, carried out in such a way that coronary perfusion is established; *c*, pericardial injections, and *d*, intra-cardiac administration.

Previous work. A combination of the intravenous and intra-arterial methods was apparently employed by d'Halluin as early as 1904, but, to our knowledge, no extensive report was issued until 1926. His technic consisted in giving an intravenous injection of a 5 per cent KCl solution in doses of 0.20 gram KCl per kilo while the heart was being massaged. Fibrillation stopped promptly. Locke's solution was then injected through an artery toward the heart and, combined with persistent massage, often led to the restoration of a coördinated beat. As the rhythm and strength of beats were apparently not satisfactory, this was followed by a 5 per cent $CaCl_2$ solution injected intravenously until the desired improvement was obtained. At the Ann Arbor meeting of the Federated Societies and again at the XIIIth International Congress of Physiology at Boston, Hooker (1928, 1929) reported success with the intra-arterial method used on dogs. The technic consisted in injecting under pressure into a carotid artery about 25 cc. per kilo of an isotonic saline solution containing 0.5 per cent KCl. This caused prompt arrest of the ventricular fibrillation, the heart coming to rest in diastole. Infusion of 50 to 125 cc. of an oxygenated isotonic saline solution containing 0.024 per cent $CaCl_2$ and in addition minute concentrations of adrenalin as a rule restored a normal sequential rhythm and a normal blood pressure. At the same meetings we demonstrated by means of moving pictures that ventricular fibrillation can likewise be promptly abrogated by intracardiac injections of 5 per cent KCl, and that normal rhythm and blood pressure are restored by intraventricular injections of 5 per cent $CaCl_2$ solution to which small amounts of heparin had been added.

In this report we outline briefly our experiences with these two methods

during the last two years, discuss the *a priori* considerations and experimental facts that led to our preference for the intracardiac method and describe in detail the technic finally adopted. It is appropriate to state at once that, while we feel convinced as a result of two years' experience that the intracardiac method is adapted for resuscitation of animals under laboratory conditions, the hope of resuscitation in man by methods so far devised is necessarily limited to cases in which the surgical possibilities at least approximate those obtainable in laboratory animals. But even in these favorable instances hope of revival is not great unless the surgeon is cognizant of the physical and physiological conditions which determine success or failure when any of the methods are employed either on animals or men.

THE CORONARY PERFUSION METHOD. The most promising method for reviving the heart *in situ* consists in establishing an artificial flow through the coronary vessels before the central nervous system has lost its ability to recover from complete anemia. It is generally believed that such perfusion of the heart *in situ* can be accomplished by inserting a cannula into a large central artery (e.g., carotid) and introducing the desired saline solutions under pressure.

Our initial experiments, 18 in number, were carried out on cats, partly as a matter of economy, partly because hope of successful revival was greater in this animal. Artificial respiration was instituted, the heart was exposed, and a cannula was placed in the innominate artery ready for connection to one of 8 different solutions, all oxygenated and kept at the same temperature (35 to 37°C.) in a large movable tank of water. Moving pictures were taken, electrocardiograms and mean pressure readings were recorded, and observations were carefully placed on record.

Our technic involved the following steps: 1. The ventricles were fibrillated by application of a faradic current, usually to the right ventricle. 2. At least 2 minutes were allowed to elapse when fibrillation of a fine character was induced and a considerably longer interval when it had an undulatory character. Without entering into extensive discussion at present, it may be stated that this precaution was reasonably adequate to guarantee that such recoveries as occurred were occasioned by the solutions injected and were not spontaneous as sometimes happens in the cat. 3. Fifty to 100 cc. of oxygenated isotonic saline solution to which varying amounts of KCl (0.2 to 1 gram per liter) had been added were injected under a pressure of 80 to 90 mm. Hg. This invariably stopped fibrillation within the span of about 1 minute. All concentrations were effective, but the larger ones required smaller volumes of infusion. 4. After complete stoppage, 50 to 100 cc. of Tyrode solution or oxygenated saline solution with $CaCl_2$ (0.2 to 1 gram per liter) was slowly injected at the same time that the ventricles were gently massaged. Recovery occurred in 18 out of 26 attempts on 18 cats.

The recovery which differed essentially from that of spontaneous recovery in the cat consisted of the following sequence of events:

a. Coördinated beats of the left, then of the right auricle, at first independent in rhythm, later in unison.

b. Feeble idioventricular beats gradually increasing in strength, persistence of heart block, auricles and ventricle beating at different tempos.

c. Resumption of a supraventricular rhythm, reëstablishment of a natural circulation of the animal's own blood and restoration of arterial pressures to 60 or 100 mm. Hg.

d. Recovery of certain portions of the nervous system—provided fibrillation had not extended beyond 12 minutes—as evidenced by return of natural abdominal breathing, tracheal movements, presence of knee-jerk and corneal reflex.

These experiments demonstrated *a*, that fibrillation in the cat's heart can be promptly abolished by administration of solutions containing an excess of potassium; *b*, that a natural coördinated beat can be re-awakened by calcium; *c*, that an efficient circulation of the animal's own blood can be restored, and *d*, that such treatment is not deleterious; on the contrary, the reflex functions of the central nervous system revive if too long an interval does not intervene.

Having established the value of the method as a means of resuscitating the smaller hearts of cats, we next applied the same technic to dogs anesthetized and prepared as were the cats. Ventricular fibrillation was produced as before, and, after a lapse of 4 to 15 minutes, oxygenated saline solutions containing different amounts of KCl were infused in varying amounts through a carotid or innominate artery under a pressure of 70 to 80 mm. Hg. Altogether 20 dogs were so treated in our first series of experiments. The infusion of such KCl solutions stopped the fibrillation only when KCl was present in concentrations beyond 5 grams per liter. A lapse of two to six minutes was required, however, before fibrillation ceased entirely. Between 200 to 500 cc. of fluid needed to be infused before this occurred. Upon complete cessation of fibrillation as determined by the absence of electrocardiographic oscillations, injections of oxygenated saline solutions containing equivalent concentrations of Ca ions were made. After a short interval, the ventricles were gently massaged, and in some instances small doses of adrenalin were administered. In this series of experiments our efforts were not successful in the sense that a normal beat and natural circulation were ever reëstablished. The ventricles often gave a number of feeble idioventricular beats in which peristaltic waves swept slowly over the surface, but these either died down or reverted to a fibrillating movement. Vigorous coördinated beats dominated by a supraventricular pacemaker never developed. In fact our results were so discouraging that the belief began to crystallize that the

method holds out no great promise of success. We therefore abandoned the method *pro temp* and proceeded to experiment with intraventricular injections to be described later on.

In the meantime, Hooker (1928) had found the perfusion method just as efficacious in dogs. It obviously became our duty to discover the cause of our failures. Another series of experiments on 17 dogs was required, however, before we were able to obtain a single successful revival, but these experiments were most valuable since they revealed to us some of the conditions and factors that apparently determine success or failure with the method. The frank discussion of these unsuccessful experiments is important for they may prove of practical value to surgeons in applying the method to man. When a fairly large amount of liquid is injected under a pressure of 70 to 80 mm. Hg it tends first to fill the rather capacious arterial system in an animal as large as a dog. Since fluid is continuously leaving the aorta by its large branches, a considerable volume continues to be consumed in this way. Furthermore, the semilunar valves are rarely efficient during the stage of fibrillation with the result that fluid leaks back and causes a pronounced distention of the left cardiac chambers. The use of so much fluid for purposes other than coronary perfusion means that more than 20 seconds may elapse and that more than 100 cc. of fluid may be injected before any material volume reaches the coronary vessels. We came to this conclusion in our earlier observations by watching the appearance of colored solutions in the coronaries, but later actually compared the coronary flow with the arterial inflow. This wastage of fluid diminishes somewhat when the arterial dead space has once been filled, but is never decreased to such an extent that the bulk of injected liquid flows through the coronary vessels. The volume of coronary flow is determined by the pressure at the root of the aorta and this in turn is a resultant of the volume of fluid entering the carotid artery under pressure and the continued outflow from the aorta by the peripheral vessels and semilunar valves. The net effect is that the animal becomes so waterlogged and the left ventricle so distended that small hope of reëstablishing a natural circulation of blood remains.

The intent and aim of the arterial infusion method is to establish an effective perfusion of the coronary vessels, thus enabling sufficient amounts of K and Ca to reach every fraction of ventricular muscle within short intervals of time. We learned, however, that, as applicable to the intact animal, the procedure falls far short of the ideal conditions existing in an excised heart in which a perfusion cannula is inserted directly into the aorta. In the latter, practically all of the fluid passes through the coronaries; in perfusing the heart *in situ* only a small part of the total fluid injected reaches the coronary system, the exact proportion depending

a, on the dead space of the arterial system, and *b*, on the size and number of branches permitting a continued drainage of the aorta.

From Hooker's preliminary report and also from personal conversations, it gradually became obvious to us that an essential difference existed in the way that the method had been employed by him and by us. We had injected large quantities of fluid (400 to 600 cc.) under pressures of 70 to 80 mm.; Hooker had injected comparatively small quantities (150 cc.) under pressures of 140 to 150 mm. Upon adopting a similar procedure we were gratified to find that the method was at least not invariably disappointing; two of seven dogs in this series reviving with the aid of cardiac massage. In the second dog, 80 cc. of 0.5 per cent KCl solution was

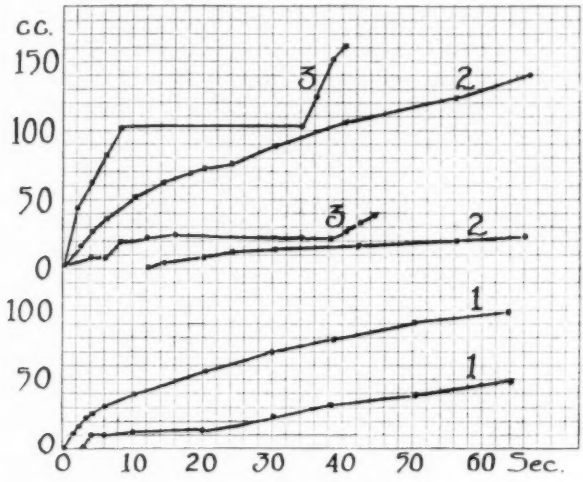

Fig. 1

injected rapidly under a pressure of 200 mm. Hg. Liquid was at once visible in the coronary arteries, and within 1½ minutes fibrillation had ceased. This was followed by 80 cc. of 0.5 $CaCl_2$ solution under a similar pressure, aided by gentle massage. No tendency to beat was obvious. Another 50 cc. of the 0.5 per cent $CaCl_2$ solution was similarly injected and with the aid of gentle massage vigorous beats were gradually restored. The blood pressure returned to normal, and reflexes could again be elicited.

A few recoveries resulting from this simple change of technic naturally led us to search for the reasons. By using colored solutions and making careful tests of a physical nature, it soon became apparent that a sudden jet of fluid sent into an empty aorta and directed toward the heart does more than fill the aortic dead space. In the dog, the orifice of the innomi-

nate artery constitutes a nozzle from which fluid introduced under low pressure largely dribbles into the arterial dead space, but, injected under high pressure, projects a stream into the root of the aorta, a part of which actually enters the coronary system. The former method is invariably disappointing; the latter is more often successful in reviving the dog's ventricles from fibrillation. In fortunate injections sufficient amounts of K and Ca salts may thus reach the coronary vessels and in so doing first stop fibrillation and later reëstablish normal beats.

Having reached these conclusions which served at least as a working hypothesis, it was necessary to support them by more exact evidence. This was done by measuring simultaneously the rate of arterial inflow and the rate of coronary flow derived from the cannulated coronary sinus. The results of the few typical experiments are plotted graphically in figure 1. The pair of curves labeled 1 show respectively the rate of inflow into the innominate artery of a cat under a perfusion pressure of 70 mm. Hg (upper curve), and the corresponding coronary outflow (lower curve). The plot shows that the arterial inflow occurs very rapidly for about 3 seconds, i.e., during the interval that the arterial dead space is being filled with fluid. After this has been accomplished, the inflow rate becomes more gradual and not until then does the coronary flow begin. Calculations of the ratio between the arterial inflow and coronary outflow show that the latter begins after approximately 25 cc. of saline have been injected. At the end of 20 seconds one-fourth of the total volume injected was recovered from the coronary vessels, while at the end of 60 seconds approximately one-half of the total volume injected passed through the vessels of the heart. No question remains but that the vessels of the cat's heart are perfused fairly efficiently by the method which probably accounts in part at least for its success in this animal.

The pair of curves labeled *2* offer a similar comparison of the rate of arterial inflow and coronary outflow in the dog. In this instance the perfusion pressure was 75 mm. Hg. Several obvious differences are shown in these plots. The initial steep rise of the arterial inflow curve (upper) lasting until the arterial system has been reasonably filled required an interval of 8 seconds, and no coronary flow (lower curve) was recorded until 14 seconds had elapsed. Comparisons of the two curves show that at the end of 20, 40 and 60 seconds respectively only $\frac{1}{10}$, $\frac{1}{8}$ and $\frac{1}{7}$ of the total fluid injected had passed through the coronary vessels, in other words, coronary perfusion is greatly delayed and never represents as large a fraction of the infusion as in the cat. Thus, after infusing a total volume of 150 cc. over a period of 60 seconds, only 25 cc. were actually measured as passing through the coronary system. The less effective perfusion of the coronaries of the dog undoubtedly is concerned with the unfavorable effects.

The pair of curves labeled *3* illustrate the effects when a similar quantity of saline is injected under high perfusion pressures; in this instance 240 mm. Hg. The lower curve shows that in each of the two injections the coronary flow increases very promptly. During the first injection 25 out of 100 cc. flowed through the coronary vessels within 16 seconds; in the second injection about 15 out of 50 cc. were likewise recovered from the coronary sinus.

These results demonstrate that a successful flow through the coronary vessels of any large animal can be accomplished only by maintaining a high perfusion pressure, and that no effective perfusion of coronary vessels obtains unless this precaution is adopted. These suggestions should be given most careful consideration by surgeons who attempt to employ this method in the resuscitation of human hearts from ventricular fibrillation.

Benefiting by these discoveries, it has been our privilege to confirm Hooker's observations that the dog's heart *can* be revived from ventricular fibrillation by successive injections of saline solutions containing dilute K and Ca salts, provided injections are given under high pressures. It remains our impression, however, that its usefulness, even in laboratory animals, is rather limited. When we consider the circumstances that normally attend death from electrocution, the procedure is obviously incommensurate with the practical needs. The ligation and sacrifice of a carotid artery is itself attended with considerable risk to cerebral function in man. Its surgical preparation and connection to bulky flasks containing sterile, properly oxygenated saline solutions is obviously out of the question in the field. Removal of a patient to a hospital or operating room loses the few vital minutes that the central nervous system can endure total anemia. On the other hand, when the method is capable of prompt application it is worthy of trial in those rare instances of ventricular fibrillation that develop while the patient is upon the operating table.

THE PREMISES UNDERLYING RESUSCITATION BY INTRACARDIAC INJECTIONS. In spite of the meager hope of success, studies upon intracardiac injections of potassium and calcium salts were energetically undertaken almost from the start on account of its possible feasibility as a field measure. We pictured to ourselves that in contrast to the carotid perfusion method injections into the ventricular chambers might prove comparatively simple in execution. No great surgical ability or anatomical knowledge is required to thrust a sterile trochar needle through the chest wall into the heart and inject a syringeful of solution. *A priori* considerations suggested that the method was worthy of extensive trial. The ventricular cavities, particularly the right, communicate through abundant Thebesian vessels with the vascular system of the ventricles. The transmission of substances from the intraventricular cavities to the ultimate muscular fractions is exceed-

ingly probable at least as long as fibrillary action continues. The coronary pressure drops to zero but the intraventricular pressure, owing to the pressure energy liberated by the incoördinate contractions remains fairly well elevated. In addition, fluid injected into the left ventricle may enter the root of the aorta and so gain access to cardiac muscle through the coronary system as well. Finally, the possibility also remained that fibrillation can be stopped and an idioventricular coördinated rhythm be redeveloped by contact of chemicals and drugs with the interior layers of the ventricles.

The experimental procedure of intracardiac injections. A number of preliminary experiments were first performed in which the approximate doses of potassium and calcium for intraventricular injections were established. In eight early experiments in which success was not attained two important requirements were emphasized as necessary for recovery, viz.: 1. The absolute necessity of ascertaining that fibrillation had entirely ceased. 2. Care that the potassium was nicely balanced but not overbalanced by subsequent use of calcium. Three of the early successful experiments may be briefly reported in the following protocols:

PROTOCOLS. *Experiment 0-31, March 2, 1928, Dog 5.5 kilos—Weight of heart 55 grams.*

1:09 Ventricles fibrillated by faradic current.
1:11½ 5 cc. KCl (5 per cent) each ventricle.
1:13 Ventricular fibrillation stopped—electrocardiogram string quiet.
1:14½ 6 cc. $CaCl_2$ (5 per cent) each ventricle.
1:17½ Ventricular massage.
1:19½ Idioventricular beats appear.
1:24 Natural auriculo-ventricular sequence established; mean blood pressure, 82 mm.
1:26½ Reversion to idioventricular rhythm.
1:34 Reëstablishment of normal a-v sequence.
1:35 Artificial respiration discontinued—heart accelerated—animal breathed—natural respiratory movements.
1:37 Mean blood pressure—120 mm.
1:38 Central vagus stimulation—mean blood pressure rose to 160 mm. Hg.
2:12 Natural rapid beat continued, blood pressure = 130 mm. Hg.
2:13 Recurrence of fibrillation, probably as a result of excessive heating from lamps used for moving picture illumination.

Experiment 0-32, March 6, 1928—Dog 5.5 kilos—Weight of heart 48.5.

10:30 Ventricular fibrillation by faradic current.
10:31½ 5 cc. KCl (5 per cent) in each ventricle.
10:36 Fibrillation completely stopped, galvanometer string quiet.
10:36¼ 5 cc. $CaCl_2$—5 per cent in each ventricle—3 manual compressions of heart.
10:37 Ventricles respond to mechanical stimulation.
10:39¾ Massage.
10:40½ Idioventricular beats.
10:46 Idioventricular beats.
10:51¾ Normal beats—natural a-v sequence.

10:59¾ Normal beats.
11:05 Normal beats—mean blood pressure = 30 mm. Hg.
11:08 Normal beats—mean blood pressure = 30 mm. Hg.—artificial respiration discontinued.
11:09 Animal breathes—blood pressure rises to 140 mm. Hg.
11:10 Artificial respiration restored—blood pressure declines to 64 mm. Hg.
11:13 Blood pressure 44 mm. Hg.—rectal temperature, 36°C.
11:15 Blood pressure 32 mm. Hg.—respiration discontinued temporarily.
11:15½ Animal breathes—blood pressure rises to 140 mm. Hg.
11:19 Artificial respiration reëstablished—blood pressure falls to 30 mm. Hg.
11:20 CO_2 content of inspired air increased so that animal just breathed. Blood pressure rose to 58 mm. Hg—heart rate slowed.
11:37 Blood pressure 64 mm. Hg.
1:00 Gradual hypodynamic condition of heart developing.
1:45 Dog expired.

Experiment 0-38, March 8, 1928—Dog 7.5 kilos—Weight of heart 82 grams.

2:50 Ventricular fibrillation by faradization.
2:51 5 cc. KCl (5 per cent) in each ventricle.
2:51½ Right auricle beating, left quiescent.
2:56 Slight continued fibrillary movements—3 cc. KCl (5 per cent) right ventricle
2:56½ Standstill of ventricles.
2:57¼ 5 cc. $CaCl_2$—with 5 mgm. heparin each ventricle.
3:00 Ventricles respond to mechanical stimulation—no rhythmic beats.
3:02½ No rhythmic beats—1 cc. 1:1000 adrenalin into left ventricle—ventricle responds to mechanical stimulation, but no evidence of rhythmicity.
3:03 Massage as indicated on drum.
3:03½ Second time followed by recovery with tremendous elevation of blood pressure = 183 mm. Hg.
3:06 Ventricular irregularity due to partial heart block.
3:10 Recovery normal rhythm—blood pressure = 74.
3:12 Artificial respiration discontinued—no respiratory response.
3:33¾ Stimulation central left vagus—no effect.
5:10 Heart fibrillated purposely second time.

These experiments demonstrated that fibrillation existing for 1½ to 2½ minutes in the dog's heart can be promptly abolished by injecting into the cavity of each ventricle about 5 cc. of 5 per cent KCl solution. After assurance is given by means of electrocardiograms that every visible sign of fibrillatory contraction is in abeyance, the subsequent injections of calcium chloride combined with massage restore a normal beat. In one instance the blood pressure remained relatively low, in the other it returned to normal levels. In both instances the respiratory and vasomotor centers gave evidence of activity and the animal survived for a number of hours, after which time it was killed. The experimental procedure established in these experiments was retained with slight modifications throughout the work of testing this method of revival more carefully. It consisted in the following preparations:

A dog was anesthetized with a small dose of morphine and sodium barbital, given intravenously. Under this anesthetic the pupillary and patellar

reflexes were present. Artificial respiration was established, the heart was exposed for observation, mean arterial pressure was continuously recorded on a kymograph, while electrocardiograms (lead II) were periodically recorded. Two 10 cc. syringes, provided with large trochar needles, were filled, one with a 5 per cent KCl solution, the other with 5 per cent $CaCl_2$ solutions. To the latter small amounts of heparin (1 mgm. per cc.) were later added in order to reduce the hazard of accelerated coagulation when such massive doses of calcium were added to the blood within the ventricle. The test consisted in carrying out the following routine:

1. The ventricles were fibrillated by application of a current from an induction coil.

2. An interval of time, ranging from 1 to 8 minutes in different experiments, was allowed to elapse.

3. At least 50 mgm. KCl per kilo were injected directly into the two ventricular cavities as a 5 per cent solution. This was accomplished by thrusting a large hypodermic needle directly through the ventricular walls, taking care of course to avoid the larger vessels.

4. Massage was avoided until all visible evidence of fibrillation had disappeared. Electrocardiographic records by lead II were watched for the complete disappearance of oscillations.

5. Five per cent CaCl solution containing 1 mgm. heparin per cc. was similarly injected into each ventricle, the dose being 50 mgm. per kilo.

6. After the lapse of approximately one minute, the heart was gently massaged until a coördinate beat was restored.

7. After recovery the following observations were made as to the reactivity of the central nervous system. The knee-jerk and pupillary reflexes were tested. The activity of the respiratory center was determined by discontinuing artificial respiration and noting the reëstablishment of natural respiratory movements, the tonic activity of the vagus center was tested by noting the effect of vagus section upon heart rate, and the response of the vasomotor center was evaluated by the effect that stimulation of a central vagus nerve had upon the mean arterial pressure.

The efficiency of the method in repeated revivals may be illustrated by blood pressure tracings from a single experiment. Figure 2 shows segments of such tracings obtained in experiment 0–70, carried out on a 10.5 kilo dog, whose heart on post mortem weighed 84 grams. At *1* the ventricles were fibrillated. The decline of blood pressure to zero is recorded on a stationary drum. At *2* (3 minutes later) 6 cc. and 4 cc. of a 5 per cent KCl solution were injected into the right and left ventricular cavities, respectively. At *3* (1 minute later) fibrillation stopped. At *4* (1 minute later) similar injections of 5 per cent $CaCl_2$—heparin injections were given. At *5* massage was started and continued until *6*. The moderate elevation of blood pressure produced by rhythmic compression of the ventricles is

shown. At *6* an idioventricular beat developed and continued to maintain blood pressure. At *7* a sinus rhythm developed and elevated the blood

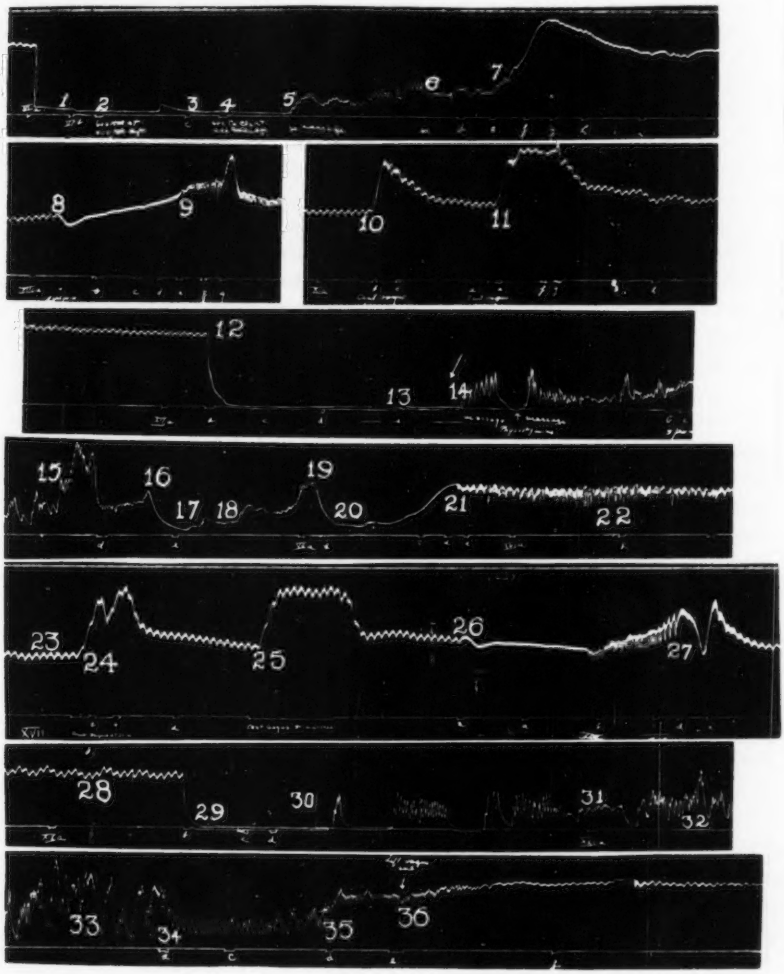

Fig. 2

pressure further, as shown toward the end of the first record. Summarizing we may say that within 8 or 9 minutes after onset of fibrillation a normal circulation had been restored. Sixteen minutes later, i.e., at *8*, artificial

respiration was discontinued. A typical rise of blood pressure due to stimulation of the vasomotor center occurred. At *9* the animal began to breathe naturally. In the third segment the viability of the vasomotor center is demonstrated by the pressor reactions at *10* and *11* which occurred as a result of stimulating the central end of the left vagus nerve. The fourth record shows how well the blood pressure was maintained approximately one hour after revival.

With such satisfactory evidence of recovery the ventricles were fibrillated a second time, the fall of blood pressure to zero being recorded on a moving drum at *12*. Three minutes after the onset of fibrillation, i.e., at *13*, 5 per cent KCl solution was again injected, 5 cc. into each ventricular cavity. While the drum was stopped at *14*, fibrillation was arrested, and 5 cc. of the $CaCl_2$—heparin solution were introduced into each ventricle. The drum was started and the heart massaged, as shown by the somewhat irregular pressure variations. At *15* spontaneous ventricular beats developed for a short time, elevating the blood pressure, but they suddenly ceased at *16* with a consequent fall of pressure. Mechanical stimulation between *17* and *18* again inaugurated a temporary period of beat, but the spontaneous beats failed again, with a resulting fall from *19* to *20*. The heart was again stimulated by mechanical taps between *20* and *21* and then developed a sinus rhythm. Many premature systoles make the blood pressure record between *21* and *22* somewhat irregular but at *23*, recorded 44 minutes after the onset of the second fibrillation at *12*, it had become quite normal. At *24* and *25* characteristic pressure responses following stimulation of the central end of the vagus nerve are shown. In addition to these tests showing the viability of the vasomotor center, the knee-jerks were exaggerated and the corneal reflex was present. Activity of the respiratory center, after discontinuing artificial respiration (at *26*), is shown at *27*. Two hours and 34 minutes were allowed to elapse. The normal character of the blood pressure is shown in the records at *28*.

The ventricles were fibrillated a third time at *29*; injection of KCl and $CaCl_2$ solutions were repeated, while the drum was stopped at *30*. Massage was continued until *31*, a point at which a short period of automatic coördinated beats developed. When these failed the heart was again massaged; and at *32* an automatic rhythm, interrupted by short periods of stoppage, occurred. At *34* the first regular and permanent idioventricular rhythm occurred, and the blood pressure rose as soon as the rhythm was taken over by the S-A node at *35*. Tests of central nervous activity were now negative, however. Thus, stimulation of the central end of the vagus at *36* proved ineffective.

Revivals of the heart after being thrown into fibrillation two or three times, such as are illustrated in detail by these experiments, were common experiences with our method.

SUMMARY. The details of all attempts to abrogate fibrillation by intracardiac injections of KCl solutions and to restore a normal rhythm by subsequent injections of $CaCl_2$ solutions need not be reviewed at this time. We shall be content to summarize the results from a practical viewpoint.

A. Potassium inhibition. 1. In 21 experiments 40 to 110 mgm. KCl per kilo injected as a 5 per cent solutions and in divided doses into the two ventricular cavities abolished fibrillation within 1 to 6 minutes (average 2.8 minutes). Massage was not required to bring about this action. Forty milligrams per kilo or less similarly introduced in eight experiments did not abolish fibrillation. We conclude that while 50 mgm. KCl per kilo may not represent the minimal effective dose it is the smallest dose that can be relied upon.

2. The complete recovery of the circulation and of reflexes in animals makes it improbable that permanent toxic effects of even larger doses of potassium persist after subsequent injections of equivalent calcium solutions.

3. Injections into the cavities of both ventricles appear to be essential in order to elicit an inhibitory effect within a reasonable interval of time. In eight experiments in which the total dose was introduced into the right ventricle alone, fibrillation was totally abolished in 6 to 19 minutes. Since the average continuation of fibrillation after such injections was over 10 minutes, in contrast to an average of 2.8 minutes after biventricular injections, we feel that this mode of administration is not of practical value on account of the needless lengthening of the period that the circulation remains in abeyance.

B. Calcium neutralization and recovery. 1. In eleven experiments 1 cc. per kilo of a 5 per cent $CaCl_2$ solution, containing 1 mgm. heparin, were administered after fibrillation had stopped completely after KCl. The ventricles were not massaged. No single instance of recovery occurred. Upon being stimulated mechanically or electrically, the ventricles gave no response. In a few experiments feeble auricular beats developed, the contraction waves spreading lazily from one portion to the other.

2. In 26 instances similar doses of the $CaCl_2$-heparin solutions were administered, but the ventricles were massaged in such a way that a material elevation of aortic pressure occurred with each compression (cf. fig. 2 at *5, 14, 30*). Within a few minutes the ventricles reacted to each compression by a beat of their own. Thereupon a spontaneous idioventricular beat appeared. Whenever this was sufficient to maintain a fair arterial pressure, a normal sinus rhythm soon developed. The conclusion was reached that $CaCl_2$ solutions are incapable of restoring a coördinated beat without the assistance of manual massage.

3. Numerous drugs were added to the $CaCl_2$-heparin solutions in still

other experiments with the hope that some agent might be found to replace massage as an agent for developing an automatic rhythm. In fact the therapeutic part of our program for the past year was directed toward a search for such an agent. We were entirely unsuccessful in our efforts.

4. Even with the use of massage recovery was by no means always attained. In 17 other tests in which the same procedure was used, no recovery resulted. In some of these experiments no evidence of ventricular irritability obtained; they neither gave spontaneous beats nor reacted to mechanical or electrical stimuli. In others, ephemeral weak ventricular contractions, incapable of expelling blood from the ventricles, resulted. In still others, a reversion to fibrillation occurred.

While the suggested procedure is far from being uniformly successful, the reasonable percentage of recoveries demonstrates the clinical possibilities of the method. However, the practical superiority over the arterial perfusion method did not prove to be as great as was hoped for. The facts that manual massage is necessary and that biventricular injections are difficult to give through the chest wall are serious obstacles to its easy application in the field. For the present, the hope of resuscitation by either method appears to be limited to fortunate cases that can be treated in the operating room.

CONCLUSIONS

1. The existence of ventricular fibrillation can be recognized with certainty only by direct inspection of the heart or electrographically.

2. Ventricular fibrillation in the normal dog's heart is a progressive condition from which spontaneous recovery does not occur.

3. Restoration of a coördinated rhythm can occur only after every vestige of fibrillary activity has disappeared.

4. The normal duration of fibrillation is generally more than 15 minutes; often it exceeds 30 minutes. As the central nervous system does not survive for such an interval of time, revival cannot be expected if fibrillation is allowed to run its natural course.

5. Fibrillation of the ventricles can be abolished artificially by use of KCl, perfused through the coronary system *via* a carotid artery and the aorta, but only provided injections are given under high pressure. Fluid injected under low pressures has a tendency first to fill the arterial dead space and then to drain from the arterial system by the large branches and imperfectly closed semilunar valves, with the result that the animal becomes water-logged before an efficient coronary circulation is established.

6. After cessation of fibrillation a coördinated beat can be restored by similar perfusion of an isotonic solution containing an excess of Ca.

7. Since coronary perfusion demands the sacrifice and ligation of a carotid artery under aseptic conditions—a performance requiring rather

elaborate perfusion apparatus, etc.—it is frequently not adaptable to practical needs.

8. Ventricular fibrillation in the dog can also be abolished promptly by injecting 1 cc. per kilo of a 5 per cent KCl solution as divided doses into cavities of each ventricle. Massage is not required to produce these effects. Injections into both ventricles, however, appear to be essential in order to produce a prompt arrest. Effective doses introduced into the right ventricle delay the arrest of fibrillation so much as to make their practical use in resuscitation of doubtful value.

9. The large doses of KCl employed appear to have no permanent toxic effect when followed by equivalent doses of calcium, as in our procedure.

10. Subsequent injection into the ventricular cavities of a solution containing 5 per cent $CaCl_2$ and 0.1 per cent heparin in doses equal to 1 cc. per kilo causes a resumption of a coördinated beat provided the fluid is pumped through the coronary vessels by massage. Without massage it is ineffective.

11. No chemical substitute for massage has been found as a result of extensive experimentation.

12. While the methods offer some promise of success when applied promptly to patients after electric shock, hope of resuscitation in man by methods so far devised is necessarily limited to cases in which the surgical possibilities and circumstances approximate those obtainable in laboratory animals. The fact that cardiac massage is required is a serious drawback to the practical use of the intracardiac method in the field.

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THE RECOVERY OF THE SPLEEN FROM CONTRACTION INDUCED BY EXERCISE

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The various factors, particularly exercise, which cause marked changes in the size of the spleen have recently been studied in the laboratory of Barcroft.[1] Several methods for observing the size of the spleen have been used by these workers, but the one which seemed most satisfactory is that in which the spleen was made extracutaneous, the so-called exteriorized preparation, and as such was kept under observation for several months. The method shows that the spleen contracts to about $\frac{1}{3}$ to $\frac{1}{2}$ of its volume on exercise, and to an even smaller size on death or severe hemorrhage. The diminution in size is apparently caused by a discharge of blood corpuscles from the spleen pulp. The data presented by these investigators implies that after the exercise the spleen regains its normal size. It seemed worth while to us to investigate the course of the recovery in detail.

The present paper deals primarily with an attempt to trace this recovery in size of the cat spleen following periods of widely varying intensity and duration.

METHODS. The technique employed by us differed from that of the Cambridge workers in three respects: the spleen was not made permanently extracutaneous, but was brought to the surface of the body only when measurements were to be made; both decerebrated and amytalized cats were employed; and exercise was induced by means of single make and break shocks or tetanizing current applied to the limbs of the animal. The method for decerebration was that devised by Schmidt (1923). As a rule very little bleeding was observed and the animals developed no rigidity. An electric heating pad was used to maintain the proper temperature following decerebration.

At least an hour elapsed before any observations were made on decerebrated cats in order to obviate the effects of the ether used in the operation. In the case of amytalized cats there usually was no muscular disturbance. The cats passed under the influence of the anesthetic without a struggle. Therefore we were able to open the animal and carry on measurements as soon as the animal had reached the operative stage.

[1] This work has been published in a series of papers in the Journal of Physiology from 1925–1929.

To observe the spleen a linear incision about 3 inches long was made through the skin about ½ inch below the costal margin. The incision was then carried through the abdominal muscles and peritoneum. The spleen could then be lifted gently through the opening and allowed to rest on the skin where measurements of area could be made when necessary. During exercise and at other times when measurements were not in order the spleen was returned to its place in the abdomen and the incision temporarily closed with clamps. Control experiments showed that the elevation of the spleen to the surface and its replacement had no appreciable effect on the size of the organ under the conditions of these experiments. The stimulus was derived from four dry cells connected with an inductorium from which electrodes were attached to the limbs of the animals in various ways.

In the manner thus described we were enabled to make observations during two or three separate periods of exercise with each of a large number of animals. This would have been impracticable had exteriorization been made permanent. Each animal, after measurements were made, was asphyxiated and the post-mortem size recorded.

In measuring the size of spleens an adaptation of Barcroft's method was used. With his exteriorized preparation he traced the outline of the spleen on a piece of celluloid and transferred the drawing to paper. In the present work we have followed the same procedure with the exception that a thin glass plate was used instead of celluloid. To determine the area of a given drawing the length was measured in millimeters and multiplied by the average of several measurements of width. By taking a sufficient number of measurements of the width a reasonably accurate approximation of the area may be obtained. This method was thoroughly checked against calculations made with a planimeter, and found to agree within 1 per cent. Barcroft et al. (1925) discussed at length the surface-volume ratio of the spleen and have shown that the volume equals approximately the 3/2 power of the area. However, unless the actual volume of blood entering or leaving the spleen is under discussion it is not necessary to consider the volume at all. Therefore we have used changes in surface as a criterion for changes in size of the organ.

In order to express results obtained with spleens of different normal sizes it is necessary to have a standard to which all cases may be referred. Since these results are all relative and not absolute, it is possible to express them in per cent, taking the normal resting size as 100 per cent. Any stimulation or exercise would cause a contraction, the extent of which might be calculated as a certain per cent of the normal. Time curves of the ordinary sort may be plotted if desired (as per cent of normal against minutes or hours) or the data presented in tabular form.

In evaluating such data, we have to consider the accuracy of the method

and sources of error. Here it must be remembered, first of all, that we are not concerned with absolute, but with relative sizes. Barcroft and Stephens (1927) have found in work where the absolute size of the spleen was of importance that the personal factor has considerable significance. Indeed, Barcroft and Stephens differed by about 14 per cent in their measurements whenever they compared results on the same spleens. Here we have obviated this difficulty by having the same one of us do all the measuring. In other words, errors which may be termed personal are constant throughout and do not affect comparative measurements.

There remain, however, unavoidable variations from one drawing to the next on the same spleen. In order to determine the extent of these the following experiment was performed. A block of wood, flat on one side, was cut to represent a typical spleen in size and shape. It was then drawn on glass, transferred to paper, and the area measured by exactly the same method as the spleen. Thirty-four drawings were made so that a good average might be obtained. This average was checked with the measurement of the block itself and found to lie within 1 per cent of the actual size. It was found that on the average the individual measurements varied from the mean by 2.5 per cent and therefore the average dispersion was 5 per cent. It is safer to double this value and consider the average dispersion to be 10 per cent. Thus we can say that any individual measurement may be trusted to within 5 per cent. Now the error may be reduced by making several tracings of the spleen whenever possible (usually four were made) and taking the average of these. In the case of the artificial spleen it was found that the mean variation of such averages was 1.25 per cent, or the mean dispersion was 2.5 per cent. If we double the values, as before,we are safe in considering them accurate to within 2.5 per cent. This means that changes of spleen size shown by the data to be greater than 10 per cent in the case of individual measurements and greater than 5 per cent in the case of an average of four such measurements may be considered significant.

One further situation should be mentioned. At the beginning of the recovery of the spleen from strong contraction the organ increases in size rapidly. Here it is impossible to get an average because the size is continually changing and we have to rely on individual points. Although the error of a given point may be taken at 10 per cent still we may discover the general trend of a recovery by drawing a continuous series of outlines while the rapid increase is going on. The areas of these tracings may afterwards be plotted as a series of points and their general trend may be represented by a smooth curve drawn through them. In this way the rate of recovery may be approximately ascertained.

Results. The results of our experiments on both decerebrated and amytalized cats confirm those of Barcroft, in that we have consistently found a considerable decrease in the size of the spleen immediately follow-

ing a period of muscular exercise. The spleens usually decreased to about $\frac{1}{2}$ to $\frac{2}{3}$ of the normal area (see table 1).

A comparison of exercise caused by single shocks given twice per second with that caused by a tetanizing current shows quite clearly that the exercise induced by short application of the latter is as effective as a much longer period of that induced by the former (see table 1). However, the slower stimulation brings about a much closer approximation to natural exercise, such as running; therefore we used primarily this method in studying the recovery of the spleen, although the course of recovery was qualitatively the same with both types of stimulus.

TABLE 1

Degree of contraction of cat spleens under various conditions

CAT NUMBER	TYPE OF ANESTHESIA	TYPE OF STIMULUS	DURATION OF STIMULUS	GREATEST CONTRACTION IN PER CENT OF NORMAL
6	Decerebrate	Tetanizing	10 seconds	71
7	Decerebrate	Tetanizing	10 seconds	75.5
9	Decerebrate	Tetanizing	30 seconds	72
11	Decerebrate	Tetanizing	2 minutes	60
13	Decerebrate	Make and break	3 minutes	72
14	Decerebrate	Make and break	3 minutes	61
15	Decerebrate	Make and break	12 minutes	80
18	Amytal	Make and break	10 minutes	67
19	Amytal	Make and break	10 minutes	57
20	Amytal	Make and break	15 minutes	55
21	Amytal	Tetanizing	15 seconds	50
31	Amytal	Make and break	8 minutes	64
32	Amytal	Make and break	2 minutes	55
34	Amytal	Make and break	3 minutes	69
35	Amytal	Make and break	7 minutes	47
38	Amytal	Make and break	6 minutes	46

At the time the first measurement of the spleen is made, recovery appears to be already in progress, and it continues until the spleen has regained its pre-exercise or resting size. Continuous measurement of the spleen at frequent intervals indicates a characteristic course of recovery which may be divided rather distinctly into two phases, a first, or rapid phase, lasting from three to five minutes, and a second phase of quite variable length, during which the spleen more slowly completes its return to normal (see fig. 1).

Considering in detail the recovery of the spleen from contraction, the most obvious feature is the very rapid increase in size the moment the exercise ceases. It usually takes from $\frac{1}{2}$ to 1 minute to reopen the incision, bring the spleen to the surface, and begin tracing the outlines. In every

case which we have observed the spleen is in the process of expanding at the time when the first measurement is made, because the first 5 or 6 tracings, representing the lapse of about 1 minute, show constantly increasing areas. If there were a latent period after exercise during which the spleen remained in a state of complete contraction all of these first tracings should have approximately the same areas. This is, however, not the case.

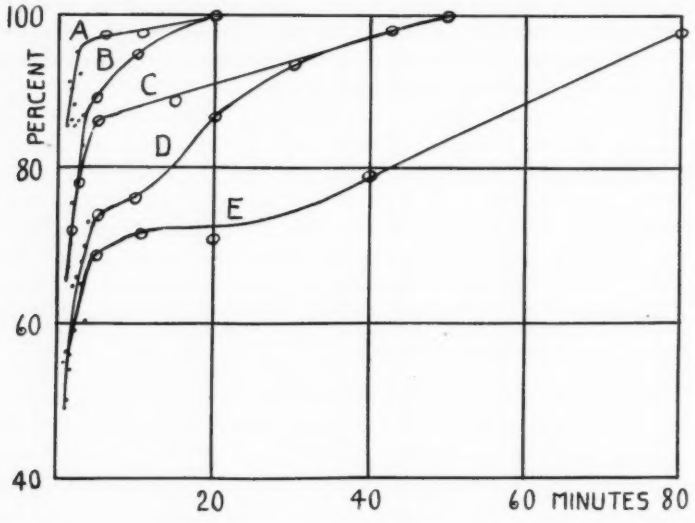

Fig. 1. Illustrates recovery curves of different types. The initial phase in all is rapid, but the second phase varies.

Curve *A:* Decerebrated. Stimulus 30 seconds. Tetanizing current.

Curve *B:* Amytalized. Stimulus 10 minutes. Induction shocks at ½ second intervals.

Curve *C:* Decerebrated. Stimulus 3 minutes. Induction shocks at ½ second intervals.

Curve *D:* Amytalized. Stimulus 12 seconds. Tetanizing current.

Curve *E:* Amytalized. Stimulus 2 minutes. Induction shocks at ½ second intervals.

It is, of course, possible that in the very short interval just prior to the first measurement the spleen may have been even more contracted than our first determination would indicate. Inasmuch as in cases of considerable exercise the areas of the first two or three tracings usually correspond to the post-mortem size of the spleen, it is unlikely that the contraction could have been very much greater than the contraction which we have found. Moreover these areas, when expressed as per cent of the normal area, correspond to a reasonable degree with similar areas calculated by

Barcroft with exteriorized spleens. We can therefore say that in our first measurements we are observing the spleen just as it starts its first phase of recovery.

This first phase of recovery continues from 3 to 5 minutes depending upon the individual case. It usually represents about 10 to 20 per cent of the normal area and within limits of experimental error was constant in all spleens studied (see table 2). From this table it will be seen that the ratio of the time to the degree of recovery in the first phase, that is to

TABLE 2

Rate of recovery during the first phase, after exercise

The rates may be expressed as the ratio $\frac{\text{time of recovery}}{\text{amount of recovery}}$ where the amount of recovery is taken as the difference in the area, expressed as per cent of the normal, between the beginning and the end of the first phase.

DECEREBRATE CATS				AMYTALIZED CATS			
Cat number	Time of recovery	Amount of recovery	$\frac{\text{Time}}{\text{Amount}}$	Cat number	Time of recovery	Amount of recovery	$\frac{\text{Time}}{\text{Amount}}$
	minutes	*per cent*			*minutes*	*per cent*	
6	4	13	0.31	18	4	15	0.26
6	5	19	0.26	18	5	23	0.22
7	5	16	0.31	19	5	17	0.23
9	4	11	0.36	19	10	23	0.43
10	3	9	0.33	20	5	25	0.20
10	4	16	0.25	20	5	15	0.33
10	3	18	0.17	20	4	12	0.33
11	3	11	0.27	21	3	25	0.16
13	5	18	0.28	31	5	15	0.33
14	5	22	0.23	32	10	29	0.34
14	4	13	0.31	32	4	15	0.27
14	4	14	0.28	34	5	17	0.39
15	5	17	0.29	34	2	10	0.20
				35	5	14	0.36
				38	5	20	0.25
Average.....			0.28				0.29

say, speed of recovery, is approximately constant, taking into consideration the individual variability of the animals with respect to age, etc.

After five minutes the rate of recovery is definitely slower but on the other hand is appreciably more variable than in the first phase. In the first place the second phase of recovery does not follow the same course in every experiment. In some cases immediately after the first phase there is a lapse of several minutes (5 to 15) during which the spleen does not change in size at all. After this delay the recovery again proceeds (see

fig. 1, curves *D* and *E*). In most cases, however, the second phase begins with no delay and proceeds at a rate which is first relatively rapid but which becomes slower as the spleen approaches its normal size. Hence it is not feasible to try to express the recovery-time relationship as a mathematical ratio (see fig. 1).

In the second place, when the duration of the exercise is varied, all other experimental conditions being held constant, the effect on the two phases is quite different. As was noted above (and see table 2) the first phase is approximately constant in extent and length of time for completion. The duration of the second phase varies very widely, particularly with those animals subjected to amytal anesthesia. Table 3 shows the results of several experiments in which animals were subjected to two periods of

TABLE 3

The relation of time for complete recovery (including both phases) to duration of stimulus

The mode and intensity of stimulus in every pair of experiments was the same.

CAT NUMBER	TYPE OF ANESTHESIA	DURATION OF STIMULUS IN FIRST TRIAL	DURATION OF RECOVERY	DURATION OF STIMULUS IN SECOND TRIAL	DURATION OF RECOVERY
7	Decerebrate	10 seconds	10 minutes	30 seconds	30 minutes
15	Decerebrate	½ minute	2 minutes	8 minutes	10 minutes
15	Decerebrate	4 minutes	5 minutes	12 minutes	10 minutes
18	Amytal	3 minutes	5 minutes	10 minutes	20 minutes
20	Amytal	2 minutes	10 minutes	15 minutes	120 minutes
32	Amytal	½ minute	25 minutes	2 minutes	80 minutes
31	Amytal	2 minutes	20 minutes	8 minutes	*150 minutes
34	Amytal	3 minutes	30 minutes	1 minute	10 minutes
38	Amytal	6 minutes	120 minutes	½ minute	20 minutes

* This experiment was only partially completed and the figure was obtained approximately by extrapolation.

stimulation (one long, the other short), the mode of application and intensity of the stimulus being kept the same. It will be seen that in general the duration of the second phase of recovery varies directly with the amount of exercise. We found this to be the case regardless of whether the short period of exercise preceded the long or vice versa.

The effect of exercise on the spleens of cats anesthetized with amytal and those decerebrated, as suggested above, may be quantitatively different. In the first place the degree of contraction when measured as per cent of resting size was greater for equivalent exercise for the amytalized cats than for the decerebrated. In the second place it is of importance to mention that the average resting size of the spleens of the amytal cats studied was more than twice as great as the average resting size for decerebrated cats; 18 experiments with the former showed an average of 33.6

sq. cm., and 15 with the latter averaged 11.1 sq. cm. It is possible, if not probable, that the nervous shock of the major operation involved in the decerebration may have contracted the spleens to some extent before observations on exercise were made. It is also possible that one effect of amytal may be to distend the spleen beyond the normal limits. To throw some light upon this point we anesthetized a number of cats with amytal, observed the usual large resting spleen and then decerebrated. We found that the decerebration, following amytal, produced practically no demonstrable effect on spleen size. We also reversed the order of the foregoing procedure, i.e., we decerebrated first and then administered amytal. The result was an increase in size of the resting spleen.

At the present time all we can say is that the presence of amytal makes for a spleen larger in size than the resting spleen of a decerebrated cat which is not under the influence of an anesthetic. The greater degree of contraction noted in amytal cats may hence be due to the fact that the contracting range of a spleen originally large and probably not contracted to any extent is greater than the range in a decerebrated cat whose spleen is probably already somewhat contracted. On the other hand, the average minimal area in the former in per cent of normal is about 20 per cent less than the latter, although in absolute units it is greater. The cause of this discrepancy has not as yet been determined. However, the general type of curve obtained is the same in both cases (see fig. 1). The first phase of recovery has approximately the same speed with both types of preparations. The average ratio of time to degree of recovery as seen in decerebrated cats, according to table 2, was 0.28. The average obtained in fifteen experiments on amytalized cats was 0.29. Although such an unusually close correspondence is probably fortuitous, still the averages must be practically identical.

The second phase of recovery is much slower with amytalized than with decerebrated cats. The average time for recovery in the latter was about 18 minutes, for the former approximately 40 minutes, although a similar variation of time of recovery with amount of exercise was noted here as in the case of decerebrated cats (see table 3).

It is noteworthy in this connection that the usual hyperpnea during and following exercise was relatively less in those cats anesthetized with amytal. In view of this sluggishness of the respiratory system it is not surprising that the recovery of the spleen of amytalized cats is retarded if we admit that the spleen is a reservoir for red blood corpuscles, and contracts in times of oxidative need for the purpose of expelling more oxygen carriers. Hence in amytal anesthesia with the oxygen intake less, the need for extra red blood corpuscles in the general circulation following exercise would continue for a greater length of time than in the unanesthetized decerebrated animals.

Our experiments show quite clearly that the recovery of spleen size depends on the duration of exercise. Although no data were obtained on oxygen consumption during exercise and the period of recovery following, it seems safe to assume that the extent of the oxygen debt would vary in direct relation to the total time for recovery. The first or rapid phase of recovery, more or less constant in all cases seems to be independent of the length or severity of exercise. Apparently some other mechanism aside from oxygen, CO_2, etc., controls this phase of spleen recovery.

Barcroft has shown that asphyxia as well as exercise causes the spleen to contract. We performed several experiments using this type of stimulus on decerebrated cats and found that the contraction and the course of the subsequent recovery was the same as has been previously discussed. In the case of the amytalized cat there is a contraction, but we have not been able to observe recovery because the animal did not survive (or recover itself from the asphyxia). This was due to the failure of the respiratory system noted above to respond to oxygen need. The amytalized cat in a state of asphyxia shows none of the usual symptoms. Its respiratory rate is not increased and there are none of the muscular contractions usually associated with asphyxia.

SUMMARY

1. Changes in spleen size following electrically induced exercise were studied in decerebrated and amytalized cats.

2. In order to trace the recovery in size of the spleen a temporary exteriorization was made.

3. It was found that there are two phases of recovery in both types of preparation. The first or rapid phase lasting approximately 3 to 5 minutes in all cases seems to be independent of the length or severity of exercise; the second or slow phase, on the other hand, varies in most cases with the extent of the exercise.

4. Recovery for equivalent exercise appeared to be appreciably slower for the amytalized cat than for the decerebrated.

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THE EFFECT OF ASPHYXIA ON THE SPLEEN OF CURARIZED AND UNCURARIZED DECAPITATE CATS

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A method has been described by Cook and Rose (1929) for observing rapid changes in the size of the spleen. They were able to follow the course of recovery of the spleen after a period of strenuous exercise or after partial asphyxia. It appeared to us desirable, however, to follow the course of the original contraction as well as the subsequent recovery. Two of the more practical methods of causing the spleen to contract are violent exercise and asphyxia. In our experiments the former was not preferable, since the motion imparted to the body by the muscular activity makes it very difficult to get accurate tracings of the spleen. The use of asphyxia is to some extent open to the same objection since during its course the animal struggles considerably. Nevertheless we have been able to overcome this difficulty by the use of curare which renders the animal motionless and makes it possible to obtain a continuous series of tracings throughout the entire contraction of the spleen. During the investigation certain results of general interest were obtained which will also be discussed.

METHODS. Cats were used throughout as the experimental animal. After a cat had been anesthetized with ether, its abdominal wall was opened and the area of the spleen determined by the method described by Cook and Rose. The incision was then closed and the cat decapitated by Sherrington's method. Decapitation was used instead of decerebration in order to cut off completely all the higher centers. Artificial respiration was employed and the body temperature was kept at approximately 37°C.

To obtain the asphyxia curve the spleen was brought to the surface, the air supply cut off, and measurements made approximately every 15 seconds for several minutes. This, of course, necessitated the exposure of the spleen to the air for some time. In order to determine whether the handling and the 5 minute exposure, per se, affected the size of the spleen a control experiment was performed with a normal spleen without asphyxia. It was found that five minutes' exposure had no significant effect on the size.

To immobilize the cat curare was administered. A dose of 1 cc. of 1 per cent curare per kilo body weight was injected into the femoral vein. The cat became entirely incapable of motion in a few minutes and re-

mained so for one to several hours after which time the responses returned. If the cat is asphyxiated during the period that the curare is effective there is no activity. The cat lies perfectly quiet and the spleen may be easily measured.

RESULTS. *1. Changes in spleen size on decapitation.* The effect of decapitation is to cause an immediate increase in the size of the spleen, (in 9 experiments the average was 20 per cent) followed by a gradual further increase over several hours (see table 1). There may be two causes for this increase: 1, the cutting off of the higher nervous centers, with its accompanying shock, and 2, the blowing off of the ether. The spleen, under ether, is always in a partially contracted state. Hence when the ether is removed it might be expected to increase in size. This increase, however, should be gradual, and should coincide roughly with the reduction of ether concentration in the blood. We find, however, that the most rapid increase takes place within a very few minutes after

TABLE 1

Prolonged effect of decapitation on spleen size

TIME	SIZE OF SPLEEN
	sq. cm.
Before decapitation	39.2
5 minutes after decapitation	43.8
60 minutes after decapitation	51.4
90 minutes after decapitation	52.6
180 minutes after decapitation	55.8
240 minutes after decapitation	54.7
300 minutes after decapitation	56.0

the ether has been removed, in other words, before any significant quantity of ether can have been removed by the artificial respiration. The initial, sharp increase then cannot be due primarily to the removal of ether. Furthermore, the slow increase, lasting several hours, continues longer than is necessary for all the ether to be blown off; therefore this also must be due principally to the effect of decapitation. Nevertheless, the effect on spleen size of recovery from the ether is in the same direction as the effect of decapitation and undoubtedly is superposed on it.

2. Course of splenic contraction during asphyxia. At the onset of asphyxia there is little or no effect, but at the end of 1 to 2½ minutes the spleen begins to decrease in size and decreases rapidly to its minimum (fig. 1). Further asphyxia, or death, fails to cause further decrease. There is considerable individual variation with respect to the slope of the asphyxia curve and to the length of time necessary for the contraction to become complete, but the general course of the reaction is quite characteristic.

In some instances the first part of the curve is nearly a horizontal line (such as might be expected if there were a true latent period) but in others the downward slope is gentle but definite from the beginning. In all cases the transition from the slow contraction to the rapid is quite abrupt and clearcut as is also the cessation of contraction when the minimum size is reached.

3. Effect of curare. Figure 1 is a composite curve showing the data of eleven asphyxia experiments, seven of which were performed under curare,

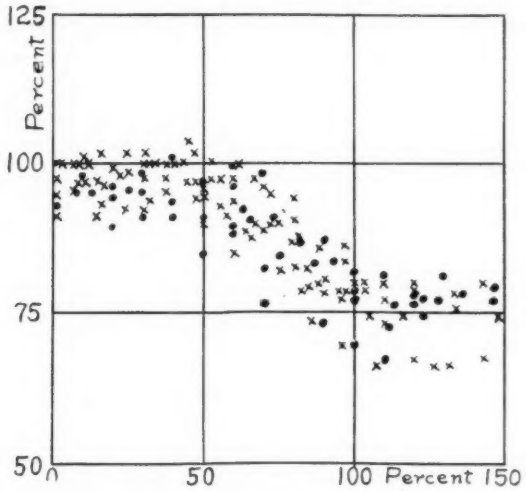

Fig. 1. A composite graph of several asphyxiations. The ordinates represent spleen areas reduced to per cent, where the normal or initial size is taken at 100 per cent. Since the time to complete the contraction varied considerably in individual cases although the course of the contraction was similar throughout, the time may also be put on a percentage basis. Therefore the number of minutes for a spleen to contract to the minimum is taken as 100 per cent and the intermediate points calculated as per cent of the time to reach the minimum. The solid circles represent untreated cats, and the crosses curarized cats.

and four without curare. It will be observed that all these curves fall within the same limits, the general trend of all is the same, and the general features of the individual asphyxia curve are preserved in the composite. There is no essential difference in the contraction of the spleen with or without curare. The following conclusions may be drawn.

A. Curare does not have the same effect on spleen muscle that it does on skeletal muscle, otherwise all spleen action would be inhibited and there would be no contraction even when the animal was *in extremis*. This is to be expected of an organ provided with smooth muscle only.

B. We have here a method whereby the spleen may be measured outside

the body during asphyxia. Since the animal cannot move the disadvantages due to struggling are obviated, and at the same time the reaction of the spleen to nervous or other stimuli remains normal.

C. Since exercise, or violent muscular effort, normally acts as a stimulus to contract the spleen, and since, without curare under asphyxia the onset of the rapid phase of contraction coincides in point of time with the violent kicking, it might be supposed that the kicking itself was the cause of the contraction. Since the response of the spleen to asphyxia is substantially the same whether the animal struggles or whether it is immobilized by curare, it follows that the muscular effort of the struggling itself is not the stimulus which causes the contraction of the spleen. The reaction under curare indicates that the skeletal muscular activity and the splenic contraction are both set off, so to speak, independently of each other at about the same stage in the process of asphyxia. One further piece of evidence tends to substantiate this view. Under amytal anesthesia, as mentioned in a previous paper (Cook and Rose) the muscular activity during asphyxia is very much reduced and sometimes entirely suppressed. Here also the typical splenic reaction to asphyxia is found: There are then three different conditions (amytalized intact, decapitate, and curarized decapitate) in all of which the spleen reacts in an essentially identical manner to asphyxia.

4. The action of the autonomic system. Barcroft and Stephens (1927) have shown that intact nerve supply is necessary for contraction of the spleen since neither muscular exercise nor asphyxia will cause the denervated spleen to contract. Both the spleen and pilo-motor muscles are innervated by the thoracico-sympathetic outflow of the autonomic system, and both function under curare as well as without curare. But in none of our experiments did the hair begin to rise in asphyxia until the splenic contraction was practically complete.

SUMMARY

1. When a cat is decapitated an immediate and considerable increase in the size of its spleen takes place.

2. When a decapitate cat is asphyxiated there is at first little or no change in the size of its spleen, but after about two minutes the spleen contracts rapidly.

3. The same effect of asphyxia on the spleen is observed if the animal has been immobilized by curare. Therefore the muscular activity of the uncurarized cat during asphyxia cannot itself be a stimulus for the contraction of the spleen.

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MUSCULAR TRAINING

I. The Physiological Effect of Daily Repetition of the Same Amount of Light Muscular Work

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It is common experience that repetition of a muscular movement increases the ability for doing that particular muscular movement. The physiological changes that produce this increase in efficiency have been studied by several observers. The majority of the experiments have been made by comparing the results on men in training with the results obtained on a group of non-athletes. This type of experiment is always open to the criticism that the physiological response of the athlete to work is an inherent property of the athletic type and the physiological differences have not been brought about by the training. When one considers the number of men that go out for athletics each year and how few develop into first class athletes, there must be more to the development of an athlete than merely the system of training, for each man receives the same training throughout the season or seasons. Hill (1925) says, "Some people are much more skilled than others. To a large degree, of course, the skill and grace associated with athletic prowess is natural and inborn, to a large degree, however, it can be produced by training and breeding."

So the comparison between athletes and non-athletes may not give the true results of training. For that reason we have followed individuals during a course of training to riding a stationary bicycle at a definite speed and load over a period of several weeks, studying the metabolic, respiratory and circulatory responses to the work. It is very true that the results of an intensive study of a few men may not be applied to a large group, yet it is true that the results obtained are due to the training and not to individual differences in men.

Method of experiment. The subject rode a stationary bicycle one-half hour each day for several weeks, keeping time to a metronome and maintaining a constant load throughout the working period. Determinations of the metabolic, respiratory and circulatory responses were made two or three times a week during this period.

On the day that the determinations were made the first subject (C. G.) came to the laboratory without breakfast in the morning, rested one-half

hour on a bed beside the bicycle, after which a five-minute sample of the expired air was collected in a Douglas bag for the subject's basal metabolism. Pulse rate, respiratory rate and blood pressure were taken after the collection of the expired air. Similar determinations were taken with the subject sitting on the bicycle ten minutes after climbing on the seat. The subject started pedalling the bicycle and pedalled for one-half hour with the brake balanced at 1500 grams. After breathing through the valve for one minute to wash out the dead space, the expired air was collected during the last three minutes of pedalling and the pulse rate was taken immediately after the exercise was over. With an oxygen consumption of 1200–1300 cc. per minute, there can be no doubt that the subject had reached a "steady state" after 27 minutes of pedalling and thus the three minute sample represented a true cross section of the oxygen metabolism due to the work. Hill and Lupton (1923) have shown that the "steady state" is reached in less than two minutes for moderate work and more recently Bock and his associates (1928) have shown in their subject, with an oxygen consumption of 2.2 liters, that a "steady state" was reached in 4 minutes and the greater part of the recovery period for light work is due to the oxygen debt acquired in the first three of four minutes of work. A graphic record of the rate of respiration was obtained by the use of a pneumograph. A fifteen second count of the pulse rate was obtained within twenty or thirty seconds after the work had stopped. The volume of the expired air was measured in a ten liter Bohr meter and duplicate samples analyzed in a Haldane gas analyzer. Each day, before the analyses of the expired air samples, an analysis was made on atmospheric air, checking 20.96 per cent for the combined CO_2 and oxygen determinations within ± 0.03 per cent (Henderson, 1918). For the second subject the procedure was essentially the same except the subject pedalled the bicycle with the brake removed at the same rate as during the work experiment. This gave a "no load" value for his metabolism and efficiency. For this determination the subject pedalled the bicycle for four minutes with the brake removed, breathed through the valve system for one minute of room air to wash out the valve system and a collection was taken for a five-minute period. The pulse rate was obtained immediately after the pedalling had been stopped.

Realizing that taking the pulse rate after the work may give no idea of the pulse during work, as Paterson (1928) has recently emphasized, various methods were tried in order to obtain the pulse rate during work, the only successful method was that of Smith (1922) using the thread galvanometer. Instead of using kaolin paste in the electrodes, green soap (Boas, 1928) was used for making contact with the body. Three electrodes were used, one to the right of the sternum in the second interspace, the second over the apex of the heart and the third was placed in contact with the abdominal wall.

The first two electrodes were connected to the galvanometer and the abdominal electrode was connected to the ground. This method gave very good results, the thread remained steady and in the camera field and was unaffected by body movements during work. In some experiments photographs were taken, in others the observer counted the number of sharp R waves for fifteen seconds, taking counts at several intervals during work. In this subject (W. B.), during very light work, the heart sounds could be heard distinctly through a stethescope and this gave a method of checking the galvanometer count by making simultaneous determinations. The determinations agreed exactly. During heavier work, however, with the noise of the moving parts of the bicycle and the rapidity of the heart beats, the auscultatory method was found to be impracticable. The photographic records show the typical response of the heart uninfluenced by any muscular movements of the subject.

This method was used during the series of experiments on W. B. The pulse rate during work was obtained using the galvanometer and during the recovery period by palpation of the radial pulse. On this subject two separate three-minute collections of the expired air were made, one from the 21 to 24 minutes and the other from the 27 to 30 minutes of working. The two collections gave results that checked from 4 cc. to 63 cc. of oxygen per minute, giving an average difference of 28.2 cc. in this series of experiments. In every other experiment in this series, the resting value was taken sitting on the bicycle instead of lying on a bed, and a ten-minute collection of the expired air was made instead of a five minute collection as in the former series.

The bicycle was designed by the senior author and was built in the Department of Mechanical Engineering of this University. It was of the prony brake type, with wooden brake bands and a cast iron fly-wheel. The balance used was graduated and sensitive to the gram, with a capacity of twenty kilograms. A screw adjustment of the brake bands controlled the tension of the bands on the drum and after the brake became worn it was found that very little adjustment was required to maintain a steady balance throughout the work.

A revolution counter attached to the brake axle gave the number of revolutions. This bicycle had a gear ration of 1:2.5. During the collection of the three-minute sample of the expired air, while pedalling, the number of revolutions for that period were recorded as well as the total number of revolutions for the half-hour of work. An attempt was made to keep the number of revolutions of the brake at 170 per minute for each subject, but this varied slightly in each case. The average number of revolutions per minute for each subject for all the experiments taken during the three-minute collection period is given in table 1, with the fastest and slowest number of revolutions in each series.

The tare weight was determined by the dynamic method used by Severinghaus (1927). This was determined each day after the experiment was finished and was found to vary slightly. These variations are given in table 2. It is noticeable that the tare weight decreased from one experiment to another; this decrease may be due to the fact that the brake became worn during the experiments which would change the tare weight. With these deviations in the speed and the tare weights, slight deviations were obtained in the total work done. Two experiments were discarded, one in the series of C. G. and one in the series of W. B. as the metronome stopped during the experiments and the number of revolutions for these

TABLE 1

Summary of number of revolutions per minute

	AVERAGE NUMBER OF REVOLUTIONS PER MINUTE	FASTEST NUMBER OF REVOLUTIONS	SLOWEST NUMBER OF REVOLUTIONS	NUMBER OF EXPERIMENTS
C. G.	168.7	171	168	11
B. P.	169.8	176	165	8
W. B.	177.8	181	172	10

TABLE 2

Summary of tare weights

	AVERAGE TARE WEIGHT OF ALL EXPERIMENTS	MAXIMAL TARE WEIGHT	MINIMAL TARE WEIGHT	NUMBER OF DETERMINATIONS
	grams	*grams*	*grams*	
C. G.	732.9	756.5	707	11
B. P.	710.5	739.5	689	8
W. B.	686.4	720.0	662	10

experiments were so different from the rest of the series that the results could not be compared.

The efficiency was calculated for several base lines, the gross efficiency in the sense of Benedict and Cathcart (1913) and the net efficiency in the first series of experiments on the basal and the "sitting on the bicycle" base lines, in the second experiment on the basal and the "no load" base line and in the third experiment on the basal, the "sitting on the bicycle" and the "no load" base lines.

The subjects were men in apparently good health, C. G. weight 80 kgm., height 180 cm., age 27 years; W. B. weight 69.5 kgm., height 176 cm., age 24 years; B. P. weight 59.0 kgm., height 170 cm., age 23 years.

Subject B. P. during a student year in Germany (1926–27) did extensive bicycling, the other two subjects have not ridden bicycles for ten or more

TABLE 3

Results of experiments on subject C. G.

DATE	BASAL VALUES O_2	BASAL VALUES CO_2	R.Q.	TOTAL VENTILATION (REDUCED TO STANDARD CONDITIONS, DRY)	RESPIRATION RATE	TIDAL AIR	BLOOD PRESSURE	PULSE RATE	WORK	E GROSS	E BASAL BASE LINE	E "SITTING ON BICYCLE" BASE LINE
	cc. per minute	*cc. per minute*		*liters per minute*	*per minute*	*cc.*	*mm. Hg*	*per minute*	*kgm. m. per minute*			
Oct. 18	263	178	0.68	4.83	11	439	115/ 86	54				
23	267	186	0.70	4.34	6	723	110/ 88	46				
25	258	180	0.70	4.61	7	658	108/ 86	45				
27	257	182	0.71	4.54	6	757	110/ 84	48				
30	267	197	0.74	4.72	6	786	114/ 70	48				
Nov. 3	266	201	0.75	4.55	5	910	110/ 90	51				
6	258	191	0.74	4.29	6	715	108/ 88	48				
8	252	180	0.72	4.17	6	695	117/ 98	47				
10	290	217	0.75	4.88	5	976	116/ 98	42				
13	268	194	0.73	4.42	5	884	114/ 92	51				
15	280	188	0.67	4.51	6	752	115/ 92	42				
Sitting on bicycle												
Oct. 18	277	202	0.73	5.99	11	545	120/ 90	67				
23	273	194	0.71	5.30	8	662	118/ 96	63				
25	271	187	0.69	5.09	8	636	114/ 90	66				
27	273	211	0.77	5.41	7	774	118/ 90	67				
30	291	219	0.75	5.64	7	805	118/ 88	61				
Nov. 3	274	206	0.75	5.07	6	645	116/ 88	60				
6	276	219	0.77	5.29	7	755	120/ 96	60				
8	262	196	0.75	5.03	7	719	120/ 98	60				
10	284	226	0.79	5.60	7	800	130/110	56				
13	272	214	0.79	5.57	7	795	120/ 98	63				
15	274	201	0.73	5.16	7	737	122/ 94	62				
Working*												
Oct. 18	1330	1118	0.84	28.30	31	913		109	385.2	13.95	17.4	17.6
20	Revolutions in this experiment increased so that it is not comparable with the rest of the series											
23	1317	1189	0.91	29.14	28	1040		100	369.9	13.42	16.8	16.9
25	1311	1096	0.84	27.46	27	1015		96	361.8	13.38	16.7	16.9
27	1306	1109	0.85	27.45	23	1193		104	371.9	13.71	17.1	17.3
30	1279	1145	0.90	27.80	25	1112		104	369.9	13.60	17.4	17.8
Nov. 3	1257	1119	0.89	27.04	27	1014		100	369.3	14.01	17.77	17.92
6	1268	1149	0.91	27.11	25	1084		104	368.9	13.82	17.4	17.7
8	1290	1092	0.85	26.33	26	1013		100	369.9	13.81	17.2	17.3
10	1286	1130	0.88	27.37	25	1095		96	369.9	13.75	17.74	17.65
13	1201	1189	0.98	27.48	24	1145		100	376.2	14.61	18.79	18.87
15	1228	1042	0.85	24.82	23	1079		100	372.6	14.60	18.92	18.80

* Pulse rate taken 20 to 30 seconds after work.

TABLE 4

Results of experiments on subject B. P.

DATE	BASAL VALUES O_2	BASAL VALUES CO_2	R.Q.	TOTAL VENTILATION (REDUCED TO STANDARD CONDITIONS, DRY)	RESPIRATION RATE	TIDAL AIR	BLOOD PRESSURE	PULSE RATE	WORK	E GROSS	E BASAL BASE LINE	E "NO LOAD" BASE LINE
1928	*cc. per minute*	*cc. per minute*		*liters per minute*	*per minute*	*cc.*	*mm. Hg*	*per minute*	*kgm. m. per minute*			
Nov. 20	215	193	0.90	4.43	5	886	106/72	52				
22	211	158	0.75	3.689	6	615	104/70	54				
24	218	159	0.73	3.796	9	422	100/68	57				
Dec. 6	240	164	0.68	4.197	10	420	96/62	52				
8	239	156	0.65	3.897	9	433	102/64	63				
13	228	161	0.71	3.668	9	408	99/62	60				
15	229	167	0.73	4.242	12	354	104/68	63				
19	248	166	0.67	4.044	11	368	110/65	59				
Working with brake removed from bicycle. "No load" values												
								PULSE RATE TAKEN IMMEDIATELY AFTER WORK				
Nov. 20												
22	452	346	0.77	7.006	8	877		72				
24	413	340	0.82	7.446	10	745		104				
Dec. 6	415	323	0.78	7.270	13	559		92				
8	414	304	0.73	6.722	8	840		92				
13	423	323	0.76	7.370	11	670		92				
15	448	351	0.78	8.164	12	680		80				
19	450	336	0.75	8.000	13	615		92				
Working												
Nov. 20	1221	1099	0.90	20.36	12	1696		135	382.9	14.91	18.1	
22	1218	1073	0.88	20.79	15	1386		136	383.6	15.05	18.2	23.93
24	1169	1026	0.88	21.25	16	1328		136	390.6	15.97	19.59	24.69
Dec. 6	1027	914	0.89	17.65	16	1103		112	373.9	17.36	22.63	29.13
8	1102	896	0.81	17.01	13	1309		116	373.9	16.51	21.07	26.46
13	1161	992	0.86	19.74	15	1316		112	399.0	16.51	20.55	25.97
15	1122	948	0.85	19.54	16	1221		116	388.9	16.69	20.97	27.81
19	1137	983	0.86	18.52	14	1323		116	401.2	16.95	21.68	28.07

B. P. did not ride on November 25, 26 and December 1.

TABLE 5

Results of experiments on subject W. B.

DATE	BASAL VALUES O_2	BASAL VALUES CO_2	R.Q.	TOTAL VENTILATION (REDUCED TO STANDARD CONDITIONS, DRY)	RESPIRATION RATE	TIDAL AIR	BLOOD PRESSURE	PULSE RATE	WORK	E GROSS	E BASAL BASE LINE	E SITTING ON BICYCLE	E "NO LOAD"
1919	*cc. per minute*	*cc. per minute*		*liters per minute*	*per minute*	*cc.*	*mm. Hg*	*per minute*	*kgm. m. per minute*				
Apr. 9	228	192	0.84	5.10	10	510	118/86	84					
10					9		116/90	70					
11	260	194	0.75	5.50	12	458	124/90	68					
15					10		116/78	76					
17	233	178	0.77	4.43	10	443	112/76	72					
20					10		113/72	76					
24	227	188	0.83	5.12	8	640	108/73	68					
27					12		114/74	72					
May 1	225	185	0.82	4.77	9	530	102/68	76					
4	228	182	0.80	5.00	8	625	112/72	72					
Sitting on bicycle													
Apr. 10	256	227	0.89	6.61	10	661		80					
15	240	218	0.91	5.93	9	660		88					
20	229	197	0.86	5.47	7	781		88					
27	260	207	0.80	6.12	12	510		80					
Pedalling bicycle with brake removed. "No load" values*													
Apr. 9	500	428	0.86	10.95	9	1216		82					
10	414	343	0.83	10.08	26	388		80					
11	459	395	0.86	10.50	24	437		72					
15	430	352	0.82	8.35	20	418		104					
17	427	324	0.76	7.97	18	443		92					
20	408	332	0.81	8.57	10	857							
24	435	378	0.87	10.16	11	923		92					
27	426	348	0.82	9.48	12	790		92					
May 1	386	320	0.83	8.55	10	855		80					
4	396	320	0.81	8.75	11	795		88					
Working*													
Apr. 9	1233	1094	0.89	26.30	36	720		138	429.6	16.62	20.38		27.95
	1229	1129	0.92	28.58					429.6	16.62	20.38		27.95

W. B. did not ride on April 21 and April 28.

* Pulse rate taken during work with thread galvanometer. Values given in this table under working were obtained about 15 minutes after work had started. See table 6 for detailed tabulation of pulse rate for this experiment.

TABLE 5—*Concluded*

DATE	O_2	CO_2	R.Q.	TOTAL VENTILATION (REDUCED TO STANDARD CONDITIONS, DRY)	RESPIRATION RATE	TIDAL AIR	BLOOD PRESSURE	PULSE RATE	WORK	E GROSS	E BASAL BASE LINE	E SITTING ON BICYCLE	E "NO LOAD"
Working*—*Concluded*													
1929	*cc. per minute*	*cc. per minute*		*liters per minute*	*per minute*	*cc.*	*mm. Hg*	*per minute*	*kgm. m. per minute*				
Apr. 10†	1265	1149	0.91	26.97	33	816		144	436.8	16.38		20.54	24.35
	1286	1153	0.90	27.65					432.0	16.16		19.95	23.56
11	1288	1149	0.89	25.87	32	818		136	433.1	16.03	20.1		24.91
	1351	1275	0.94	29.05					440.3	15.35	19.0		23.25
15	1182	1048	0.89	23.09					426.0	17.18		21.55	27.0
	1190	1051	0.88	23.16	18	1280		140	426.0	17.10		21.43	26.78
17	1237	1071	0.87	24.07	19	1266		144	417.3	16.09	19.82		21.57
	1182	1031	0.87	22.91	20	1146			417.3	16.84	20.97		26.36
20	1144	1017	0.89	22.01	16	1375		132	417.3	17.39		21.75	27.04
	1140	1020	0.89	22.62	18	1256			417.3	17.45		21.84	27.18
24	1129	959	0.85	23.85	18	1324		116	405.8	17.31	21.67		28.16
	1100	915	0.86	22.63	18	1258			398.9	17.42	21.95		28.82
27	1187	983	0.83	22.40	20	1120		120	425.2	17.34		22.20	27.05
	1238	1046	0.84	25.26	20	1263			431.8	16.84		21.32	25.68
May 1	1084	948	0.86	22.21	22	1010		112	417.8	18.52	23.37		28.76
	1141	994	0.87	23.67	20	1184			421.4	17.70	22.05		26.75
4	1184	1030	0.87	23.26	22	1057		116	413.6	16.74	20.73		25.16
	1142	987	0.86	23.31	22	1059			411.9	17.33	21.66		26.53

† Subject felt tired and stiff after riding.

years. The experiments on C. G. and B. P. were made in the fall of 1928, those on W. B. in the spring of 1929. C. G. and B. P. were in fairly good general muscular condition from active outdoor summer vacations at the start of the experiments while W. B. had taken very little exercise during the winter preceding the experiment. During the experiments the subjects did not indulge in any other type of muscular activity outside of the usual routine work of the day.

RESULTS. The results are given in tables 3, 4 and 5 and in graphic form in figures 1, 2 and 3.

In the three subjects the total metabolism for the standard work showed a gradual decrease. In subject W. B. an initial increase in the metabolism was observed that was probably due to the fact that the subject was stiff and sore from the previous ride. This stiffness and soreness disappeared after the third day. The total decrease in each case was just

beyond the limits of experimental error for in the case of W. B. an average deviation was obtained of 28 cc. per minute with a maximum deviation of 63 cc. between two samples taken during the same experiment. The decrease in the metabolism between the first and the last determinations

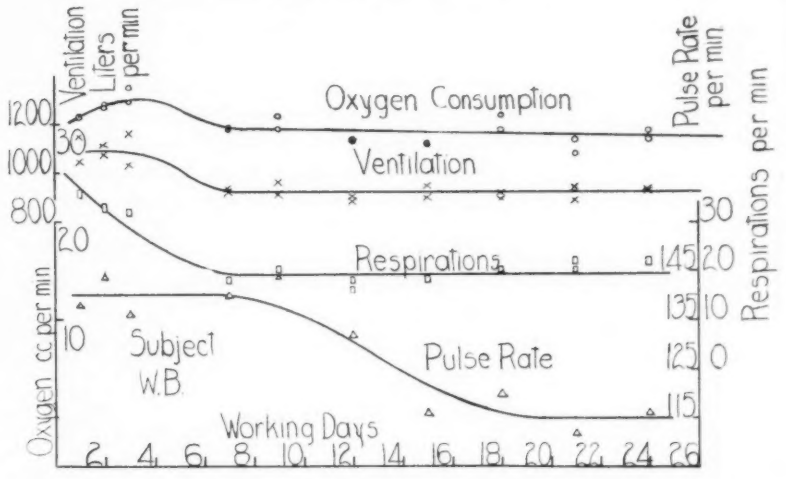

Fig. 1. Changes in oxygen consumption, ventilation, respiration and pulse rate during work in training in subject W. B.

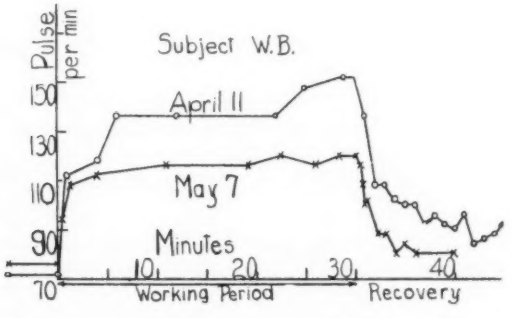

Fig. 2. Pulse rates during work and recovery before and after training

in the series of experiments on C. G. was 101 cc. of oxygen per minute, for B. P. 84 cc. and for W. B. was 91 cc. With this decrease in the total metabolism there was a decrease in the total ventilation and the number of respirations, although in the case of B. P., the number of respirations per minute remained fairly constant throughout the whole period. The per-

TABLE 6

Pulse rates taken during work and the recovery period. Subject W. B.

	WORKING DAYS									
	1	2	3	7	9	12	15	18	21	24
Before work	82	78	72	88	80	88	76	80	72	76
DURING WORK										
minutes										
1			112		124		108		104	108
2							104			
3								112	104	
4			128				108			112
5					136			116		112
6			136					120	104	
7		126		140						
11										116
12			136					120	116	
15		144			144				112	
16						132	116			
17	138			140						
19									116	116
20								124		
21					144					
22			136	140			112			120
23		138								
24								128		
25			148					120	116	
26										116
27							120			
28								124		120
29			152							
AFTER WORK	RECOVERY PERIOD									
minutes										
1	120	144	136		134			104	120	100
2		120	108		104			88	82	88
3		105	108		100			90	84	88
4		102	102		100			88	80	80
5		99	100		95			88	86	84
6	80		100		96			84	80	80
7			92		92			84	80	
8			96		92			80	80	
9			92		92			80	80	
10			90		92			84		80
11			96		88					
12			84		88					
13			86		92					
14			88		88					
15			92		88					

centage decrease in the total ventilation was greater than the percentage decrease in the oxygen consumption. Since the pulse rates in C. G. and B. P. were taken after the work and although the values obtained on W. B. with the galvanometer showed that the pulse rate during the last few minutes of work was practically the same as that taken fifteen seconds after the work, no conclusion will be drawn from the earlier experiments. The change in pulse rate for W. B. is shown graphically in figure 2. During the first two experiments on W. B., only two or three readings were taken during work but in the third determination on the third day of working, several readings were taken during work and the recovery period. The results are given graphically in the upper curve of figure 2. This method was used in several later determinations and the results on the last day of work are given in the lower curve of figure 2. The marked change in the reaction of the heart to the same amount of work is apparent following the repetition of the work each day for twenty-four days. Not only

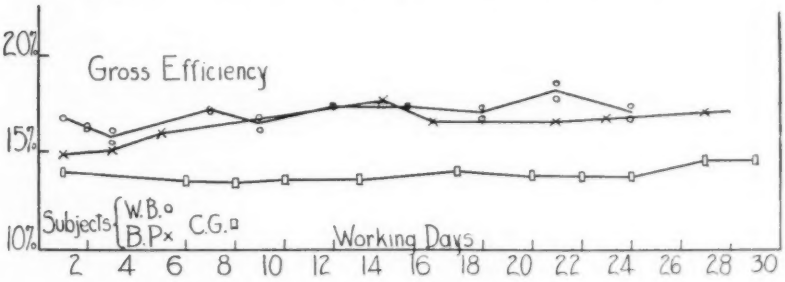

Fig. 3. Changes in gross efficiency in the three subjects during training

is the total increase during work less but the recovery period is much quicker. The pulse rates obtained after fifteen minutes of work are shown graphically in the lower curve in figure 1 for each day on which the determinations were made, and all the pulse rates determined in this series are given in table 6.

No marked or constant change was noticed in the determinations made during rest in the basal condition. A slight decrease in the metabolism of W. B. during the "no load" determination is apparent.

With the decrease in the metabolism there was a small increase in the efficiency, the plotted values for the gross efficiency being given in figure 3. The greatest increase in the gross efficiency was seen in the case of B. P. increasing from 14.91 per cent to 16.95 per cent, while C. G. only increased from 13.95 per cent to 14.60 per cent and W. B. from 16.65 per cent to 17.33 per cent. The net efficiencies calculated on the various base lines also showed slight increases. It is interesting that of the three subjects,

B. P. was the only one that had done extensive bicycling within recent years which may account for his greater increase in efficiency.

The R. Q. in nearly every experiment increased from the resting and "no load" values but never reached the value of 1. With an oxygen consumption of 1200 to 1300 cc. per minute, these experiments would compare with the experiments of Best, Furusawa and Ridout (1929) on mild exercise in which the R. Q. of the total metabolism during work increased over the resting value but did not reach 1.

DISCUSSION OF RESULTS. It is commonly assumed and there is some evidence that training enables a man to make muscular movements with the minimal expenditure of energy (Bainbridge, 1923, page 151). A. V. Hill (1925) summed up the problem of training by the following statement: "Fundamentally the teaching of athletics for anything but the shortest distances consists in training the performer to carry out the same movements at a given speed for a smaller expenditure of energy."

The earlier workers on this subject, Gruber (1891), Schnyder (1896), Burgi (1900), measured the output of carbon dioxide during a course of training and came to the conclusion that training decreased the metabolism for a given amount of work. As we now know that the amount of carbon dioxide given off during work is more an indication of the total ventilation and the formation of acid metabolites than of the total metabolism, these earlier experiments will not be considered further.

A critical survey and summary of the earlier work on the effect of training on metabolism is given by Durig (1911). In his summary of the work of Zuntz on walking, it is noticeable that the metabolism experiments were not made at the same speeds before and after the training period, so it is difficult to conclude anything concerning the effect of training on metabolism from these experiments.

The earlier experiments of Zuntz and Schamburg (1901) on the metabolism during walking were better controlled and these showed a definite change in the total metabolism as well as the experiments of Benedict and Murschhauser (1915) and Smith (1922). It is interesting to notice that in one of Smith's experiments, E. D. B., after a three weeks' absence, had a metabolism for walking 65 meters per minute higher than the value at the beginning of the exercise, yet by the second day after his return it had dropped to the average value. This sudden change in the metabolism is seen in several of the experiments, for at 55 meters it was 0.603 calories per minute on October 16 and 0.510 calories on October 18 for the same amount of work. Disregarding these sudden changes there is a gradual downward change in the metabolism in all the experiments excepting the two highest speeds and Smith concludes that lack of practice was a factor in the energy requirement of walking.

The majority of the experiments of Benedict and Cathcart (1913) were

made on M. A. M., a professional bicyclist, who was in good training at the beginning of the experiments. Yet the efficiency of M. A. M. is only slightly higher than the efficiency for the other subjects, some of whom were out of training to bicycle riding (p. 125) and some even without any previous bicycle training. Since the determinations on the untrained subjects were so few in number, Benedict and Cathcart did not draw any conclusions concerning the effect of training in this work.

Lindhard (1915) reported large changes in the total metabolism of work after a period of training to bicycle riding. Lindhard's experiments are not comparable to ours for we were interested in the changes brought about by working at a definite speed and load and doing the work each day, in Lindhard's experiments the speed and load were varied over a large range and the subject only exercised intermittently.

Atzler and his associates (1923) give an interesting graph (fig. 8, p. 39) showing the effect of training on metabolism, but since they determined the metabolism by a carbon dioxide method some of that change may be due to overventilation.

Crowden (1928) showed striking changes in the excess oxygen consumption of a worker wheeling a wheel barrow at a definite load and rate at different stages of training, claiming that the acquirement of skill in balancing the barrow is one of the results of training which accounts for the marked decrease in oxygen consumption.[1] The numerical values for the above described experiments are given in table 7.

The gradual decline in the total metabolism in our experiments is similar to that observed in the change of metabolism in the walking experiments. Durig (1911) divided training into two factors, one that is due to the increased skill and that due to the increase in the mechanical power of the muscle itself. In our experiments, riding a stationary bicycle, the very small change in the total metabolism during work would seem to indicate that the skill factor was at a minimum and the work factor was not heavy enough to bring about any changes in the muscles themselves that would increase their mechanical power. Experiments are now in progress measuring the changes in the metabolism during training to heavy work to see how great a part the work factor plays in changing the total metabolism of work. It would also be interesting to measure the metabolism of a subject untrained to bicycle riding during his early struggles on a moving bicycle and later when he is fully trained, to see how great a rôle the skill factor plays in training.

[1] Since this paper was sent to the press, an article by Schneider and Ring (THIS JOURNAL, 1929, xci, 103) has appeared in which they studied the respiratory exchange and breathing during training to light muscular work of a general nature in two individuals. For 6000 foot pounds of work they found a 4 per cent increase in the metabolism in one individual and a 6.5 per cent decrease in the second.

TABLE 7

Summary of the effect of training on the metabolism during work on man in which the determinations have been made on the same individual before and after the training period

SUBJECT (B = before training; A = after training)		DATE	METABOLISM DURING WORK	UNITS	RATE OF WORKING	PER CENT CHANGE OF METABOLISM	OBSERVER	TYPE OF EXERCISE
					meters per minute			
I	B	May 5	1048	cc. oxygen per minute	75.22	−17.4	Zuntz and Schumburg (1901)	Walking
	A	May 26	866.5		76.38			
II	B	May 1	1195.4		76.92	−13	Zuntz and Schumburg (1901)	Walking
	A	July 5	1040		75.04			
Kolmer	B		2.222	Calories for 1 kgm. weight and 1 meter walk		−0.7	Zuntz et al. (1906)	Walking
	A		2.207					
Müller	B		2.623			−11.6	Zuntz et al. (1906)	Walking
	A		2.319					
Loewy	B		2.447			0	Zuntz et al. (1906)	Walking
	A		2.469					
I	B	Nov. 26	1015	cc. oxygen used per minute	75.3	−31.5	Benedict and Murschhauser (1915)	Walking
	A	Dec. 23	695		75.9			
II	B	Mar. 16	815		75.3	+5.7		
	A	Apr. 29	864		76.9			
J. L.	B	Mar. 18	2015	cc. oxygen used per minute	683 kilogram meters per minute	−18.6	Lindhard (1915)	Bicycling
	A	Apr. 11	1639		649			

E. D. B.	B	Oct. 30	0.496	Increase in gram calories per horizontal kilogram meter	45	−11.6	Smith (1922)	Walking
	A	Dec. 7	0.439		45			
E. D. B.	B	Oct. 9	0.603		55	−22.7	Smith (1922)	Walking
	A	Apr. 1	0.466		55			
E. D. B.	B	Oct. 16	0.510		65	−4.2	Smith (1922)	Walking
	A	Mar. 30	0.489		65			
E. D. B.	B	Oct. 22	0.494		72	−10.2	Smith (1922)	Walking
	A	Dec. 3	0.444		72			
E. D. B.	B	Oct. 27	0.498		77	+1.0	Smith (1922)	Walking
	A	Apr. 10	0.505		77			
A.	B		3960	Excess oxygen used for work in cc.		−52.0	Crowden (1928)	Wheeling a wheel barrow
	A		1900					
C.	B		3060			−64.0	Crowden (1928)	Wheeling a wheel barrow
	A		1100					
C. G.	B	Oct. 18	1330	cc. oxygen used per minute	385.2	−7.7	Gemmill, Booth and Pocock	Bicycling
	A	Nov. 15	1228		372.6			
B. P.	B	Nov. 20	1221		382.9 kilogram meters per minute	−6.8		
	A	Dec. 19	1137		401.2			
W. B.	B	Apr. 9	1233		429.6	−7.3		
	A	May 4	1142		411.9			

The changes observed in our experiments were more circulatory and respiratory than in the total metabolism. The total ventilation decreased in all subjects and the number of respirations in two of the subjects showing a greater efficiency of the respiratory mechanism. The most interesting change was that seen in the pulse rate of W. B., the only subject in which pulse rate determinations were made during work. The slower pulse rate and the quicker return to normal after work of the trained man in comparison to untrained men during work has been recorded by several observers (Lindhard, 1915; Cook and Pembrey, 1912-13; Henderson, et al., 1927; Bock and associates, 1928), and has been made the basis of tests of physical efficiency by Schneider (1920) and Hambly and his associates (1922), but has not been demonstrated to the authors' knowledge on a single individual doing a definite amount of work each day with the determination taken during work.

From our experiments it is evident that training to light muscular work brings about an increase in the efficiency of the cardiac and respiratory mechanisms rather than a change in the efficiency of the muscle. This increase in the efficiency of the respiratory and cardiac mechanism allows one to do the same amount of work with fewer subjective symptoms of distress and fatigue. This change may be brought about by changes in the cells for if the oxidative mechanism of the cell is speeded up as has been suggested by Hill and Lupton (1923) and Bock and his associates (1928) the cell would be able to take a greater percentage of oxygen out of the blood circulating through the muscle and thus the demand on the circulatory and respiratory mechanisms would be diminished. This could also be brought about by an increase in the effective surface of the capillary bed supplying the muscles. Krogh (1917) considers that the quick rise in the curve is due to the overflow of the impulses from the motor area to the cardiac and respiratory centers in the brain. Gasser and Meek (1914) have shown that the acceleration of the heart at the beginning of exercise is due to decrease in tone of the cardio-inhibitory center. The pulse curve before and after training rises along the same lines (fig. 4) in each case but does not reach the same height after training as it did before. So the changes brought about in this experiment may be due to changes in the nervous control of respiration and circulation during work. The importance of the increased efficiency of the circulatory system brought about by training is stressed by Buchanan (1909) and more recently by Law (1929).

Benedict and Cathcart (1913) discussed the different types of base lines in their study of the metabolism during bicycling. Theoretically the "no load" base line is the best for that takes into account the viscous elastic effect (Dickinson, 1928) of the leg muscles while rotating the pedals, the frictional losses in the chain and sprockets and the metabolism of sitting

on the bicycle, the output of energy that has no equivalent in the measurement of the output of energy on the brake system. In class room experiments on first year medical students, it was noticed that this "no load" value was excessively high and it is seen that in W. B. this value decreased with training. Thus this base line changed throughout the series of experiments and is unsuitable for comparative efficiency determinations. The basal and the "sitting on the bicycle" determinations were more constant and are better suited for comparative studies. The most suitable efficiency for making a comparative study is the gross efficiency with the subject in the basal condition at the start of the experiment. Here we are equating the total input of energy to do a particular amount of work with the total amount of work. One might argue that sitting on the bicycle is just as essential a part of riding the bicycle as rotating the pedals by the legs so the oxygen consumed for sitting on the bicycle should not be subtracted from that used during the working period, and thus net efficiency values on "sitting on the bicycle" base line have no meaning.

SUMMARY

1. A study was made of the effect of the repetition of a definite amount of work on a stationary bicycle at a constant load each day on three subjects' metabolic, respiratory and circulatory responses over a period of several weeks.

2. The total metabolism during work showed a slight but definite decrease in the three subjects with a corresponding increase in the gross and net efficiencies.

3. The total ventilation decreased in the three subjects and the rate of the respirations in two, in the third subject the rate remained practically unchanged.

4. In subject W. B. the pulse rate was obtained during work by means of a thread galvanometer and the pulse rate showed a marked decrease during work as the experiments progressed, and with a quicker return to normal after work was over.

5. No significant changes were observed in the basal values of metabolism, blood pressure, pulse rate, total ventilation or number of respirations.

6. Training to light muscular work on a stationary bicycle brings about an increase in the efficiency of the respiratory and cardiac mechanisms rather than a marked change in the total metabolism during work.

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THE PLACENTAL TRANSMISSION OF INSULIN FROM FETUS TO MOTHER[1]

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In 1911, before Banting isolated the active principle, insulin, from the pancreas, Carlson and Drennan conducted a series of experiments purporting to show that the internal pancreatic secretion was transmitted from fetus to mother. When complete pancreatectomies were performed on gravid animals, late in pregnancy, the resultant maternal surgical diabetes was controlled to a significant extent by the theoretic passage of this pancreatic hormone from fetal blood through the placenta into the maternal circulation. The well-developed fetus has a pancreas rich in islet cells, and in fact was used as a source of insulin during the early days of insulin investigation.

Diabetic women are frequently sterile and if they do become pregnant, they usually abort. Occasionally the opportunity is afforded to examine histologically the pancreas of a new-born infant, whose mother had diabetes mellitus during her gestational period. At such an examination, the islets of Langerhans are observed to be enormously enlarged and hypertrophied as compared with those present in the infant born of a normal mother. Are these enormous islets an evidence of the attempts of the fetus in utero to furnish its diabetic mother with insulin? (Fig. 3.)

The assertions made by Carlson and Drennan were logical assumptions based on indirect evidence. The research reported in this article was an attempt to obtain conclusive proof of the placental transmission of insulin by the injection of commerical insulin into the bodies of the feti in utero and the concomitant observations of maternal blood-sugar levels.

METHOD OF PROCEDURE. Healthy pregnant goats far advanced in pregnancy were the objects of the experiment. Blood was drawn by jugular venapuncture every fifteen minutes during the course of each experiment, in order to observe any significant fluctuations in the maternal blood-sugar. Blood-sugar estimations were made according to the Folin-Wu technic. After the determination of the normal blood-sugar level of each goat, ether anesthesia was induced. With anesthesia established, a low midline

[1] Read before the International Congress of Physiology, Boston, August 21, 1929.

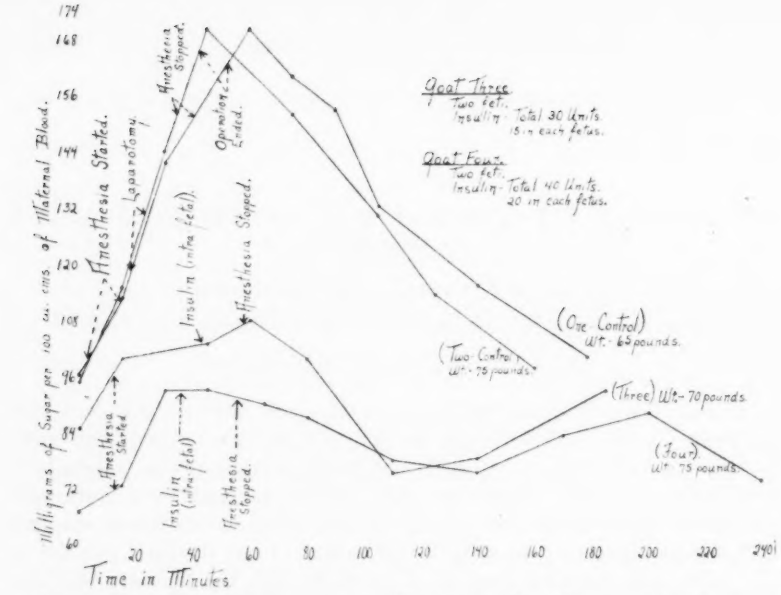

Fig. 1. Maternal blood sugar curves, illustrating the influence of insulin transmitted from the fetus to the mother.

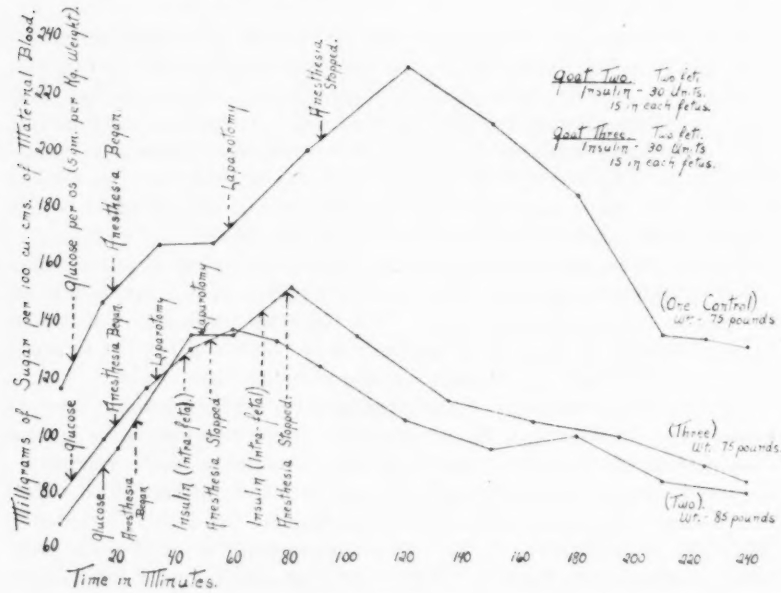

Fig. 2. Maternal blood sugar curves, following the intra-oral administration of glucose, and showing the influence of insulin transmitted from the fetus to the mother.

laparotomy was performed, the uterus palpated and the feti counted. Then using a long finely-bored needle plugged with sterile vaseline, insulin was injected through the uterine wall into each fetus with as little trauma as possible. No insulin was lost in the peritoneal cavity, and the surgeon felt reasonably certain that the entire amount was introduced intrafetally. Immediate layer closure of the abdominal wound was followed by the with-

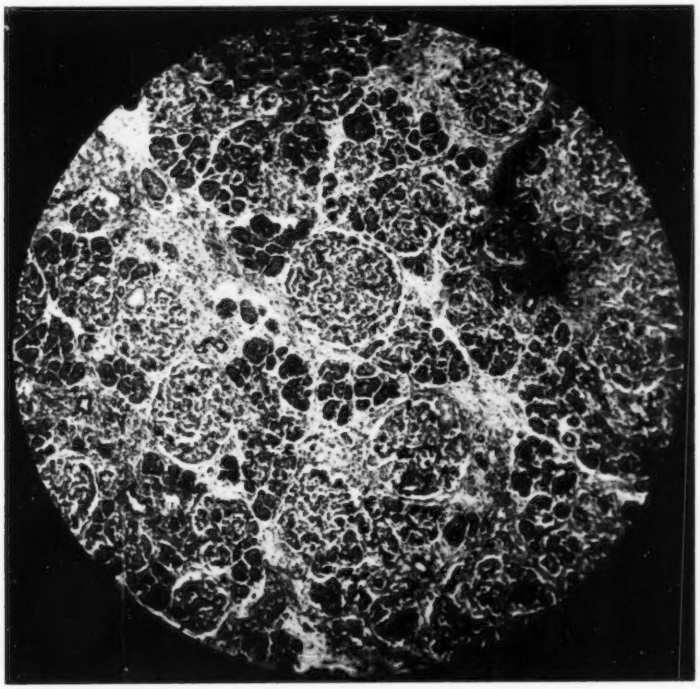

Fig. 3. The pancreas of a twelve-day-old human infant, whose mother had severe diabetes during her pregnancy. The hyperplastic islets of Langerhans suggest unusual activity on the part of the fetus in producing insulin. (Courtesy of Dr. Wilmar Allen, Hartford, Conn.)

drawal of ether. Aseptic precautions were used in every case and all the animals survived the operation serveral months, although they always went into premature labor sooner or later and gave birth to dead feti, which had probably died from hypoglycemic shock. The elicitation of fetal heart sounds by abdominal auscultation and the perception of fetal movements by palpation insured the continued life of the feti beyond the immediate period of experimentation.

Ether anesthesia elevates the blood-sugar concentration to the upper limit of normality and even beyond the renal threshold so that glycosuria may occur. By a comparison of the controls with the experiments in which insulin was injected, it can be seen that in the latter cases the maternal blood sugar never rises above the normal average level. Insulin has far greater potency in reducing hyperglycemia to normal than it does in converting the normal blood sugar to hypoglycemic levels. On this account, it was believed that an experimentally produced hyperglycemia of alimentary origin would furnish a more delicate indicator of insulin action. Accordingly five grams of glucose per kilogram of body weight of pregnant goats were given by stomach tube and the sugar-tolerance control obtained on several animals (see control-fig. 2).

The results in both series of experiments were informative and significant. There is some disparity in the initial readings of the control and experimental animals, but no more than the limits of normality permit. The ascents of the blood-sugar curves behave similarly in both series of experiments, i.e., with and without glucose, and one can readily perceive that the intrafetal injection of insulin is shortly responded to by a lowering of the maternal blood sugar.

The most likely probability is that insulin is transmitted through the placenta from fetus to mother; a remote possibility is that the fetal blood-sugar is so lowered by the insulin that glucose leaves the maternal blood to supplant this deficiency.

CONCLUSION

Insulin is transmitted through the placenta of the goat from fetus to mother, as determined by variations in maternal blood-sugar levels following intrafetal injection of commercial insulin.

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EFFECTS OF BILATERAL VAGOTOMY ON BLOOD PRESSURE AND HEART RATE

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In a previously published paper (Reed, 1925) it was shown that bilateral vagotomy in the dog produced changes in blood pressure and heart action that were partially independent. Because the results were obtained in experiments undertaken primarily for another purpose, it was determined to repeat these experiments with no other procedures that might complicate the results. Just as this work was begun Thompson and Dick (1928) published a paper in which it was shown that the changes in blood pressure and heart action following vagisection in dogs under morphine are independent, thus confirming our earlier report. These authors reviewed the literature to some extent, quoting a number of reports confirming their results.

However, it was deemed expedient to carry out the work as planned. With the completion of thirty experiments, it was apparent that the more carefully controlled and uncomplicated technic produced results quite similar to those obtained in the earlier work. Hence it was decided to combine the results of the two investigations and work out a statistical analysis of some relationships not previously considered.

Table 1 shows the condensed effects on blood pressure and heart action of bilateral vagotomy performed in the early stages of ether anesthesia. From this it will be seen that blood pressure was increased in approximately 85 per cent of the experiments, while the heart rate was increased in 58 per cent, unchanged in 12 per cent and decreased in 30 per cent.

Some text-books of physiology do not recognize any other factor involved in the rise of blood pressure following vagisection than the increased heart rate resulting from liberation of the heart from tonic inhibitory impulses, in spite of the repeated demonstration of relative independence of vasomotor and cardiac changes. (Pavlov, 1879; Reed, 1925; McDowell, 1924; Marshall, 1926; Tappan and Torrey, 1926.) Others do not stress the possibility of vasomotor involvement, which leaves with the student an impression that the increase in rate with consequent increase in cardiac output is the sole factor in producing an increased blood pressure after this operation. This table alone is sufficient proof of the importance of

recognition of at least another factor. It was clearly demonstrated that, even under the most favorable conditions, with healthy, vigorous animals, and the most careful technic, blood pressure does not invariably increase (in 15 per cent of the total series); and in 12 per cent of the experiments there was not only no increase but actually a pronounced and definite decrease. Furthermore, an examination of the graphs and detailed tabulations shows that there is an even greater discrepancy than is apparent from these tables for there is practically no correlation between the two factors, even when the changes occur in the same direction. A large change in blood pressure may accompany a slight change in heart rate and vice

TABLE 1

BLOOD PRESSURE	HEART RATE						Total
	+		0		−		
	Number	Per cent	Number	Per cent	Per cent	Number	
+	18	54.5	3	9+	7	21+	28
0	−	−	1	3+	−	−	1
−	1	3+	−	−	3	9+	4
Total...	19		4		10		33

TABLE 2

BLOOD PRESSURE	HEART RATE						Total
	+		0		−		
	Number	Per cent	Number	Per cent	Number	Per cent	
+	19	23+	5	6+	6	7+	30
0	6	7+	2	2.4	1	1.2	9
−	19	23+	11	13.4	13	15.8	43
Total...	44		18		20		82

versa, indicating that when they are of the same order, there must be an involvement of more than one factor. Byrne (1924) has shown that the fall in pressure after the administration of pilocarpine is independnet of the heart rate.

In table 2 are shown the condensed results of a much larger series of experiments in which the animals were allowed to lie under ether anesthesia for at least an hour and a half before vagotomy. In this series, every possible combination occurs, and, in the individual experiments, *almost* every possible variation of a given combination occurs. Blood pressure rose after vagotomy in 36 per cent of the experiments as against

85 per cent in the first series, while heart rate increased in 53 per cent as compared to 58 per cent in the experiments where vagotomy occurred early in the period of anesthesia. It might be argued that the number of experiments in the first series is proportionately too small. But even after granting that point, it is apparent that blood pressure is affected to a far greater degree by prolonged ether anesthesia than is heart rate. This point is sufficiently clear without further detailed analysis. Thus it is apparent that vasomotor and cardiac effects of vagisection do not, in the dog, necessarily follow, closely, any law of correlation. Reduced force of the heart beat may cause a fall in blood pressure without change of rate. That this is not the only depressor influence was shown by Marshall (1924).

That alterations in the reaction of the blood will affect cardiac activity has long been recognized. That such alterations in reaction may result from respiratory changes is obvious. One might justifiably inquire, then, whether respiratory modifications due to vagisection might be responsible for the variations in circulatory responses. However, it is possible to develop a condition in the dog, when anesthetized with ether by tracheotomy, in which only slight respiratory modifications occur after vagisection. This was done in a sufficiently large number of experiments to indicate that this factor is of minor importance in producing the responses described.

It was suggested in the earlier paper that the vasomotor center may receive, via the vagi, tonic pressor impulses that vary in intensity at times. Section would remove these impulses if there happened to be any passing at the moment, and so would lower the tonus of the center itself. If there were no such impulses present at the moment of section, then no change would occur in blood pressure, regardless of the immediate cardiac effects. In either case, subsequent possible vasotonic impulses would be prevented from reaching the vasomotor center and the final result would be depression of blood pressure. Whether these tonic impulses would represent the "vago-pressor" reflex suggested by McDowell (1924) cannot, of course, be determined from this investigation. That depression occurs most commonly after a certain degree of shock has been induced, and so, pressure in the great veins has been reduced, offers support to McDowell's hypothesis. But on the other hand, a pressor effect often follows vagisection even when a profound degree of shock exists. This fact seems to support the senior author's previous hypothesis, that the mechanism of production of depressor effects after bilateral vagotomy is to be found in the interruption of tonic impulses to the vasomotor center over fibers in the vago-sympathetic trunk.

That the condition of anesthesia is an important factor in producing the results even when vagisection is done in the early stages, is conceded. Cannon and Lewis (1927) have demonstrated that the capacity of the

heart to respond to accelerator influence is altered by anesthesia so that a heart deprived of inhibitor influences tends to beat at a "physiological maximum." But if the rate does not increase after removal of vagal impulses then it must be concluded that it is already beating at a temporary maximal rate, in which case the heart must have been desensitized by the ether to such an extent that it cannot respond at a true maximum to accelerator impulses.

Rutherford (1870) reported that no increase in blood pressure occurred after vagotomy if internal blood vessels were constricted and vice versa. Neither of these claims is confirmed by the present work. Both high and low initial pressures have been followed by both kinds of responses.

That variations in arterial pressure within wide ranges do not affect the heart rate was demonstrated by Martin (1895).

In addition to the above observations, it seemed advisable to test the state of irritability of the vagal trunks with graded tetanizing currents. In 8 dogs, approximately 7 per cent of the total number, simultaneous stimulation of the peripheral ends of both vagi with very weak tetanizing currents produced an increase in both heart rate and blood pressure. Each animal was tested carefully many times and the response was invariable. When the two trunks were stimulated separately, it was found that this pressor response was obtained from each trunk alone in three experiments, while in two instances it was obtained in both to an equal degree. When the reversal occurred on one side alone, stimulation of the other trunk produced typical vagal inhibitory effects but only after the current had been appreciably increased. When the reversal occurred, if the current was increased, the augmentation and pressor effects disappeared, to be replaced by typical inhibition and depression. When the current was again reduced, augmentation and pressor effects followed. In 6 experiments (5 per cent), stimulation of the peripheral vagi with weak currents produced a rise in blood pressure without any increase in heart rate but with decided augmentation in force.

Thus approximately 12 per cent of this large series of dogs, unselected except insofar as was necessary to insure healthy, vigorous subjects, responded to peripheral vagus stimulation in a way quite contrary to what is ordinarily described as typical of this procedure in the dog. Adequate precautions were taken against the spread of current to the adnexa and the point of stimulation was always high above the inferior cervical ganglion. Compression of the nerve trunk abolished the response. Furthermore, let it be recalled that strong currents produced the opposite effects. If the unusual results were due to accidental spread of current to sympathetic fibers, it would be expected that still more pronounced effects in the same direction would result from stronger stimulation, rather than a reversal.

About the time of Schmiedeberg's demonstration of the main path of the

accelerator fibers in mammalia, Schiff (1878) reported the existence of accelerator fibers in the vago-sympathetic trunks. Tigerstedt (1893) quoted several reports confirming Schiff. Subsequently, Arloing (1896) and Tulgan (1824) presented still further confirmation. Dale, Laidlaw and Symons (1910) investigated exhaustively this phenomenon of reversal in the cat and ferret and described in detail the conditions necessary for its production. They reported that it occurred rarely in the dog and in very weak form. In the cat, preliminary administration of nicotine was employed. It was proven that the reversal was due neither to spread of current nor to stimulation of recurrent sympathetic fibers and the conclusion was reached that it was due to stimulation of fibers of the vagus trunk itself which may be "*a*, pre-ganglionic autonomic fibers like the inhibitors; *b*, post-ganglionic fibers having their cell station in the jugular ganglion or the ganglion of the trunk, or, *c*, fibers which are normally sensory in function but capable, on artificial stimulation, of producing acceleration by antidromic action." The latter possibility was practically ruled out by these investigators in later experiments. Cannon (1929) states that he has observed this reversal in cats under ether alone, but never in dogs.

We have used no drug to produce paralysis of inhibitor fibers unless, indeed, the anesthetic itself accomplished this. To the best of our knowledge the phenomenon has not previously been reported in dogs without previous administration of a drug that would produce at least partial paralysis of inhibitor fibers.

Stewart (1918) states that usually the vago-sympathetic trunk in the dog carries some cardio-accelerator fibers. However, Gaskell (1920, p. 34) stated that, without exception, the cardio-motor fibers arise from the thoracico-lumbar divisions of the autonomic system. He presents a possible explanation of these aberrant responses (p. 82) in his theory that originally certain cells gave rise to axons which divided into two fibers, one of which was motor or inhibitory to non-striated cardiac muscle, the other to striated cardiac muscle, and that the disappearance of the former in higher forms resulted in the loss of its motor or inhibitory fibers, leaving vagus fibers inhibitory to heart muscle and sympathetic fibers augmentatory. This suggests, therefore that we are dealing with a vestigial mechanism in the few animals that show reversal.

Kuntz (1929) has assembled the evidence indicating that sympathetic fibers often take origin in the superior cervical ganglion and descend in the vago-sympathetic trunk. That variations exist in motor and inhibitory control of the gastro-intestinal and respiratory systems has been well established.

Ordinarily, simultaneous stimulation of both vagus and sympathetic fibers with the same current first produces inhibition and later acceleration. From this it is concluded that inhibitor fibers are more irritable and have a

shorter latent period than accelerator fibers. While inhibitory effects usually predominate, the reverse may occur (Franck, 1876). If our results are to be explained on the basis of Kuntz' scheme, it must be assumed that in these particular instances, for some reason, the alleged accelerator components of the vagus trunks are in a low threshold of irritability that makes possible accelerator responses while the current is still subminimal to the inhibitory mechanism. But when a stronger current is used, inhibitory effects predominate and mask the accelerator responses just as is ordinarily the case. That these unusual responses have not been more generally observed and recorded may be explained on the assumption that the optimal state of irritability is not commonly present, and that the very weak current necessary is rarely employed. It is admitted that in the experiment in which the first of these reversals occurred, the weak current was employed quite accidentally.

SUMMARY

1. Earlier observations of the effects of bilateral vagotomy have been extended and the variable vasomotor and cardiac effects noted have been analysed in more detail. In a large percentage of dogs, this operation results in a fall in blood pressure with *a*, no change in heart rate; *b*, acceleration, or *c*, retardation. When a pressor effect results from vagisection, it may be in combination with any one of these three phases of heart rate.

2. The postvagotomy effects on blood pressure, whether pressor or depressor, are not wholly dependent on cardiac action, or on respiratory influences.

3. The removal of tonic inhibitory impulses to the heart is not alone responsible for the postvagotomy pressor effect.

4. It is suggested that the postvagotomy depression of blood pressure is partly due to removal of the path by which tonic impulses reach the vasomotor centers, regardless of whether such impulses are present at the moment of section, and irrespective of their origin.

5. Stimulation of the peripheral ends of sectioned vagal trunks with a very weak current produced, in a small percentage of dogs, cardio acceleration, sometimes without any change in blood pressure, sometimes with a small rise. The mechanism of reversal is discussed in some detail.

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STUDIES IN THE NUTRITION OF THE WHITE MOUSE

VI. The Experimental Production of Xerophthalmia in Mice

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During a recent study of the experimental production of rickets in mice (1929), we observed, in addition to the rachitic changes, at 7 and 14 days, a definite xerophthalmia between 50 and 60 days in all the animals on the low phosphorus-high calcium diet of Osborne, Mendel and Park (1923). This finding was a surprise to us, since the senior author was unable, in a previous study of this question, (1925) to produce the characteristic eye disease in mice under conditions of diet which are customarily used with rats in vitamin A studies. The diet used was composed of the following ingredients: extracted casein (or extracted casein, Harris, or coagulated and extracted egg white, 31); starch, 38; crisco, 24; salt mixture (Osborne and Mendel), 7; dried yeast, 100 mgm. tablet daily. These three diets were shown to be free from vitamin A since loss of weight and xerophthalmia were produced in rats by their use. In the experiments with mice on these diets, with a concentration of 31 per cent purified protein, which lasted from 90 to 100 days, no evidence of xerophthalmia was observed in any of the 25 animals studied.

The question of vitamin A deficiency in mice seems to have been little studied in the past. Spinka (1923–24), however, in studying the effect of ultraviolet rays upon the accessory food substances, was able to produce keratomalacia in about 50 days in his control animals.

It seemed desirable. in view of the divergent results obtained above, to make a further study of the experimental production of xerophthalmia in mice. We therefore began our experiments to determine primarily the cause of failure to produce the disease in our original experiments. From the results to be reported in this paper we found that one ingredient of our original basal diet, namely crisco, contained some vitamin A, and also that the requirements of rats and mice for the protective action of vitamin A against xerophthalmia were probably quantitatively different.

Experimental. Young mice were used throughout this study using the same technique as has been described in the previous papers of this series. All the data obtained will be found in table 1.

TABLE 1

GROUP	NUMBER OF MICE	DIET	DURATION OF FEEDING TEST	AVERAGE BODY WEIGHT		REMARKS
				Beginning	End	
		gm.	*days*	*gm.*	*gm.*	
1	17	Edestin*..... 31 Starch..... 59 Salt mixture (high Ca; low P)..... 7 Dried yeast..... 3	50–60	10.1	14.4	3 died; 15 developed xerophthalmia, of which 3 were cured with cod liver oil
2	6	Edestin..... 18 Starch..... 55 Crisco..... 20 Salt mixture (Osborne and Mendel)..... 7 Dried yeast 200 mgm. daily Cod liver oil 2 drops daily	50–60	9.7	27.0	Control group
3	5	Casein..... 31 A and B free Starch..... 42 Crisco..... 20 Salt mixture..... 7 200 mgm. dried yeast daily 1 drop 5 per cent Vigantol daily	80–90	9.3	22.0	1 died. No evidence of xerophthalmia in any animal
4	10	Edestin..... 18 Starch..... 55 Crisco..... 20 Salt mixture..... 7 200 mgm. dried yeast daily 1 drop 5 per cent Vigantol daily	80–100	10.5	17.5	3 died. Rough coats. No evidence of xerophthalmia
5	6	Casein..... 31 A and B free Starch..... 42 Crisco (aerated). 20 Salt mixture..... 7 200 mgm. dried yeast daily 1 drop 5 per cent Vigantol daily	50–70	9.5	15.0	Rough coats. Two died. Definite xerophthalmia in 5 animals about 50–55 days on diet. 3 cured with cod liver oil

TABLE 1—*Concluded*

GROUP	NUMBER OF MICE	DIET	DURATION OF FEEDING TEST	AVERAGE BODY WEIGHT		REMARKS
				Beginning	End	
		gm.	*days*	*gm.*	*gm.*	
6	9	Edestin..... 18 Starch..... 55 Crisco (aerated). 20 Salt mixture..... 7 200 mgm. dried yeast daily 1 drop 5 per cent Vigantol daily	60–70	10.0	16.7	Rough coats. All died. Definite xerophthalmia in 6 animals. Swollen eyelids in 2
7	15	Edestin..... 18 Starch..... 75 Salt mixture..... 7 200 mgm. dried yeast daily 1 drop 5 per cent Vigantol daily	50–60	9.7	17.0	Definite xerophthalmia 50–55 days in 13 mice; 7 cured with cod liver oil; 6 died

* Commercial edestin (Eimer & Amend) used throughout.

RESULTS. *Group I.* This group of mice was used in the preceding study of this series, which was concerned with the production of rickets in mice. Definite evidence of the eye disorder occurred between the 50th and 60th days of the experiment, thus confirming the results of Spinka in this connection. The chief point of difference between the diet used here and the original one was a lack of fat. At once we had a suspicion that the crisco we used in the original diet might have contained some vitamin A in spite of the fact that xerophthalmia was produced in rats when it served as one of the ingredients of the diet. We used crisco in the first place because it is a hydrogenated fat (a hydrosterolein), and hence would probably contain no vitamin A.

From the above results it was evident that the fat we used must have retained some vitamin A, and we then began to make a study of its action.

Group 2. This group was used as the control, with a concentration of 18 per cent edestin. It has been shown (1925) that 31 per cent of casein is necessary to furnish enough cystine for the normal growth of the mouse, but, since edestin contains about 1 per cent cystine, a concentration of 18 per cent gives a growth curve comparable to the larger amount of casein. Commercial crisco was used to supply the fat content of the diet. Growth was excellent in all cases.

Group 3. We next desired to repeat the original experiment, using the

same diet. The casein was freed from vitamins A and B by alcoholic extraction and heating in a current of air. This diet again produced xerophthalmia in rats, but not in mice. The evidence points to the fat and also shows that the requirement of the mouse for antixerophthalmic substance is probably much less than that of the rat.

Group 4. The diet for this group contained 18 per cent edestin with 20 per cent crisco. No evidence of xerophthalmia was observed. Since this diet, without the fat, did produce the disease it is evident that crisco contains some vitamin A.

Group 5. Casein A and B free, with commercial crisco, aerated at 140°C. for 16 hours, to free it from vitamin A. Definite xerophthalmia was produced between 50 and 55 days. Three animals were cured with cod liver oil. Thus by heating and aerating the fat in this diet we made it vitamin A-free, and thus produced the disease.

Group 6. This is a repetition of the experiment of group 5, using edestin and aerated crisco. Six of the 9 animals developed the eye disorder, while the other 3 only showed inflamed and swollen eyelids.

Group 7. This group was fed the 18 per cent edestin diet, without crisco. Thirteen mice developed xerophthalmia, of which 7 were cured with cod liver oil. The remaining 6 were allowed to die.

Growth was very poor in all experimental groups reported above.

The exact method used in the manufacture of crisco is unknown to us,[1] but it is evidently a hydrogenation process which leaves a small amount of vitamin A, enough to prevent the eye disorder in mice but not in rats. In experiments of vitamin A deficiency in mice, it is recommended that one either omit the fat (i.e., crisco) altogether, or carefully destroy its vitamin A content.

CONCLUSIONS

Mice have been fed on adequate diets containing casein, extracted casein (A and B free), and edestin, as the protein factor, with commercial crisco, and the same aerated for 16 hours at 140°C., as the source of fat. Other diets omitted the crisco. The results obtained seem to justify the following conclusions:

Commercial edestin contains no vitamin A.

Commercial crisco contains enough vitamin A to prevent xerophthalmia in mice raised on a vitamin-A-free, but otherwise adequate diet, but not

[1] Since this was written we have been informed by the Proctor Gamble Co., that crisco is made from selected cottonseed oil which is purified by various refining methods, partly hydrogenated to a degree which gives it the plastic properties desirable in baking, and finally deodorized at an extremely high vacuum in an atmosphere of steam. Before packing it is chilled on brine rolls and beaten into a homogeneous mass in which form it appears on the market.

enough to protect rats from this disease, under similar conditions of diet and treatment.

The requirements of rats and mice for the antixerophthalmic factor are quantitatively different, the latter requiring less than the former.

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